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NITRIFYING POWER OF SOME PHILIPPINE SOILS

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INTRODUCTORY

This investigation deals with a certain biological property of Philippine soils. The following points of importance, directly concerned in crop production, are considered and made the basis of this investigation: (1) The correlation between the nitrifying capacity and the crop-producing power of some of the Philippine soils on which most of the important crops, such as rice, sugar cane, tobacco, and coconut are grown; (2) the comparative study of the nitrifying power of soils from different parts of the Islands; (3) the comparative study, under similar laboratory conditions, of the average nitrifying capacity of some of the Philippine soils, of the United States soils, and of the Hawaiian Territory soils; and (4) certain factors influencing the nitrifying power of some of the Philippine soils.

The old ideas regarding soil fertility have greatly changed since the development of the science of soil microbiology. According to earlier views, the decomposition of organic matter, the production of humus, and the conversion of nitrogen into nitrate in the soil were due to chemical action. Liebig in 1840 pointed out that the fertility of the soil was essentially dependent upon chemical processes. However, this view of Liebig was opposed by Pasteur, who demonstrated that biological action was mostly responsible for the decay and decomposition taking place in the soil. Some forty years ago the science of soil microbiology was yet in its infancy, and during its rapid development

numerous researches were conducted. The results from those classical experiments have shown that biological action is a dynamic force in soil fertility. After several years of experimentation, several investigators have come to the general conclusion that there exists a definite relationship between the nitrifying capacity and the crop-producing power of the soil.

Brief reference to a few of these investigations, which are considered fundamental in connection with this work, is here made.

HISTORICAL

Boussingault⁽⁴⁾ of France was the first to recognize the value of nitrification in relation to the fertility of the soil. Some years later, F. Löhnis⁽²⁰⁾ pointed out the close correlation between nitrification and the crop-producing power of the soil. Löhnis based his conclusion on the results of an experiment with a standard solution to which various soil extracts were added. After a certain period of incubation, the cultures were analyzed, and differences in nitrate production were noticed.

Vogel,⁽²⁶⁾ working with some German soils, found that the nitrifying power of the soil was directly correlated with its fertility; he therefore considered the importance of this power with regard to soil analysis.

Burgess,⁽⁷⁾ working with nine Hawaiian surface soils, all of which had been under cultivation to sugar cane and were soils of different fertility, reported that the degrees of nitrification, ammonification, and nitrogen fixation were in accordance with the fertility of the soils tested. Among the three biological tests, nitrification was found to give the most reliable results. The ammonification test was not suitable for differentiating the average Hawaiian soils except, in extreme cases, between very poor and very rich soils.

Brown,^(5, 6) upon testing the ammonifying and nitrifying powers of several plots, treated and untreated, found the results to be in direct correlation with the fertility of those plots. The results of the analysis also agreed with the crop yields. Gainey,⁽¹¹⁾ working with some Kansas soils, also found that nitrification has a direct bearing on the productivity of the soils.

Waksman⁽²⁷⁻³³⁾ of New Jersey found that, when ammonification was conducted in solution, the plot of higher fertility gave higher results in the absence of phosphates, but in the presence of phosphates reverse results were obtained. When the tests were conducted in the soil, it was noticed that acid soil of low

fertility had a greater accumulation of ammonia. He pointed out that the accumulation of ammonia in the soil after a certain period is not at all an index of soil fertility but merely indicates how incapable a certain soil is of transforming ammonia into nitrates.

On further study, he stated that in any bacteriological test of the soil, where results are sought in correlating the bacterial activities with the crop-producing power, a knowledge of the influence of the chemical conditions of the soil upon the microorganisms is indispensable. He demonstrated that, when ammonium sulphate was used for the nitrification test of an acid soil having a poor buffer action, the activity of the nitrifying bacteria was depressed. In a well-buffered acid soil, however, the use of ammonium sulphate enhanced nitrification. By using dried blood of higher quality or at high concentration (say 1 per cent) in a well-buffered soil, nitrification proceeded normally; but in a poorly buffered soil nitrification was depressed. He suggested, therefore, that for nitrification purposes ammonium sulphate should be used in the presence of a theoretical amount of calcium carbonate (210 milligrams for 30 milligrams nitrogen as ammonium sulphate) to neutralize all the nitric and sulphuric acids formed in the decomposition of ammonium sulphate. Dried blood or any nitrogenous organic materials of high quality, 0.1 per cent concentration at ten to fifteen days' incubation, or 0.25 per cent concentration at fifteen or fifteen and thirty days' incubation, should be used.

The results of the nitrification tests conducted on some experimental plots showed very conclusively that there is a definite correlation between the crop-producing power of the soil on the one hand and the number of the microorganisms and the nitrifying power of the soil on the other, due consideration being given, of course, to the effect of liming in the case of an acid soil.

Waksman, after a careful study of the effect of fertilizers upon the numbers of bacteria, reported that sodium nitrate stimulated the growth of bacteria and actinomycetes, but depressed fungi; that ammonium sulphate, making the soil acid, favored the growth of fungi and depressed the action of bacteria and actinomycetes; however, ammonium sulphate favored the growth of bacteria and actinomycetes when applied with lime. He concluded that the number of microorganisms in the soil when determinations are carried out under proper conditions, provided due allowance be made for the variability of the methods

used and of the soils, can serve as one function for measuring the bacteriological conditions of the soil and the crop production.

Noyes and Conner, (22) studying the nitrate, nitrification, and bacterial content of five typical soils, noticed that the addition of calcium carbonate increased the nitrifying power of the acid soils tested; that fertilization increased the nitrification but not so much as did calcium carbonate; that the soils that had been saturated with water for ten months contained no nitrate and, when incubated with ammonium sulphate, nitrification did not take place.

The findings of J. G. Lipman and associates of New Jersey; E. J. Russell, H. B. Hutchinson and associates of Rothamsted, England; C. Hopkins, A. L. Whiting and associates of Illinois; W. P. Kelley of California; G. P. Given of Pennsylvania; and S. F. Ashby of England confirmed the general conclusion that there is a definite correlation between the microbiological activities and soil fertility.

SOILS AND METHODS OF SAMPLING

Samples representing the average types of soil devoted to sugar cane, rice, tobacco, abacá, and coconut were taken from different parts of the Islands. Most of the samples of sugarcane soil were taken from Occidental Negros, which at present is the largest sugar district of the Islands. The samples from Occidental Negros were secured by me, through the courtesy of the sugar planters, and the samples from the other districts were obtained by the station men, by special arrangement with the plant-industry division of the Bureau of Agriculture. These samples were taken as follows:

Borings 16 centimeters deep were made with an auger in places where the soil is of the same general appearance as that of the whole field. At this depth the soil in one hectare would weigh 2,000,000 kilograms. At least fifteen such borings were made on every hectare of each field. The soils from these borings were mixed thoroughly and sampled in the usual manner. About 2 kilograms of each sample thus obtained were put in a soil bag, which was labeled and forwarded to the laboratory. According to Lipman and Martin's (17) results, soil samples obtained by the auger method, as described above, and those obtained by the aseptic method (from a flamed vertical wall of a pit) showed insignificant differences only, in bacterial

counts and in ammonifying, nitrifying, and nitrogen-fixing powers.

EXPERIMENTAL

Preparation of soil samples.—All soils were air-dried, then passed through a 20-mesh sieve, and kept in 2,000-cubic-centimeter glass bottles, stoppered with corks. For chemical analyses the samples were passed through a 60-mesh sieve and kept in small glass bottles, also provided with corks. For nitrification purposes fresh samples were used, thus eliminating any factor that might affect the viability of the organisms concerned. According to Allison,⁽²⁾ storage and desiccation affect the numbers and activities of the soil organisms.

Nitrification.—Weighed portions of air-dried soil, each equivalent to 100 grams of water-free soil, were introduced into Erlenmeyer flasks of 300 cubic centimeters capacity. The carefully weighed materials for treating the samples were added to and thoroughly mixed with the soil. Water was then added to bring the moisture content of the sample up to 60 per cent, the amount recommended by Russell, Jones, and Bahrt⁽²⁵⁾ as the best for purposes of nitrification. The mixture was stirred to insure thorough distribution of moisture. Each flask was loosely stoppered with a cork. The treated samples were incubated at 28° C. for twenty-eight days. The moisture content was kept constant during the period of incubation. The soil samples were treated as follows:

1. No treatment given (control).
2. With 0.2 gram ammonium sulphate.
3. With 0.2 gram ammonium sulphate, and calcium carbonate slightly in excess of the amount required to neutralize the theoretical amounts of nitric and sulphuric acids produced during the decomposition and nitrification process of ammonium sulphate.
4. With copra cake equivalent to 34.8 milligrams, and 37.5 milligrams of nitrogen.
5. With copra cake equivalent to 34.8 milligrams, 37.5 milligrams of nitrogen, and calcium carbonate slightly in excess of the amount required to neutralize the acid reaction of the soil.

Nitrate determination.—Devarda's method, modified by Whiting⁽³⁴⁾ and associates, was used for the nitrate determination. The water-free samples were placed in 400-cubic-centimeter shaker bottles and 300 cubic centimeters of approximately 0.5 per cent hydrochloric acid were added to each sample. The

mixture was then shaken in a mechanical shaker for from one to three hours and allowed to settle overnight. Five grams of sodium peroxide were placed in an 800-cubic-centimeter Kjeldahl flask. Two hundred cubic centimeters of the acid extract of each sample were measured out and poured on the 5 grams of sodium peroxide and then placed in an 800-cubic-centimeter Kjeldahl flask. The contents of the flask were boiled down to 20 or 30 cubic centimeters, to expel any ammonia present. Two hundred cubic centimeters of nitrogen-free distilled water were then added, together with 0.5 gram Devarda's alloy (50 per cent aluminium, 45 per cent copper, and 5 per cent zinc), and the mixture was distilled for forty minutes and then collected in a flask containing standard sulphuric acid.

Total nitrogen.—The Kjeldahl method, modified to include nitrate nitrogen,¹ was used for the determination of the nitrogen of all the samples under investigation.

Determination of acidity (Hopkins's⁽¹²⁾ method for soil acidity.—One hundred grams of air-dried soil were placed in a 400-cubic-centimeter, wide-mouthed bottle, and 250 cubic centimeters of normal potassium nitrate solution were added. The bottle was stoppered and its contents continually shaken for three hours in a shaking machine and then allowed to stand overnight. One hundred twenty-five cubic centimeters of the clear supernatant liquid were drawn off and boiled for ten minutes, to expel the carbon dioxide. It was then cooled and titrated against a standard sodium hydroxide solution. The titration number was multiplied by 2.5 (the factor worked out by Hopkins) to give the acids present in 100 grams of dried soil.

A brief description made at the time of sampling each soil under study is here given. This description is important, because of the relation between certain field conditions and crop production. It was pointed out by Löhnis and Fred⁽²¹⁾ that any biological soil test has full value only when greenhouse and field experiments are taken into consideration. From the results of the experiments, I found that the conditions and nature of the soils, the kind and time of treatment, and the kinds of crops grown have a direct bearing upon the nitrifying capacity of the soil.

¹ Described in Bulletin 107 of the Association of Official Agricultural Chemists.

LOCATION AND DESCRIPTION OF SOIL SAMPLES

BATANGAS PROVINCE, LUZON

Laboratory No. 6.—Duras, Muson, Valentin's orchard. Planted to mandarin trees, productive since 1924 but not productive before that time. No fertilization. Surface soil, clay loam 49 centimeters deep. Slightly rolling. Good drainage.

Laboratory No. 5.—San Isidro, Santo Tomas, Mrs. Feliza's orchard. Planted to mandarin trees, very productive, healthy. No fertilization. Slightly rolling. Surface soil, clay loam, 62 centimeters deep. Good drainage; under cultivation for thirty to thirty-five years.

Laboratory No. 1.—Tanauan Citrus Station, block A. Planted to mandarin trees 20 years old, productive in 1919–1920 but practically barren now. No fertilization. Slightly rolling. Surface soil, clay loam, about 36 centimeters deep; under cultivation for about thirty-five to forty years.

Laboratory No. 2.—Tanauan Citrus Station, block B, east. Planted to mandarin trees 20 years old, bore light crops since 1919. No fertilization. Slightly rolling. Surface soil, clay loam, 38 centimeters deep; under cultivation for thirty-five to forty years.

Laboratory No. 3.—Tanauan Citrus Station, block B, west. Planted to mandarin trees 20 years old, bore light crops since 1919. Light application of complete fertilizer in 1923–1924, the last application in May, 1924. Slightly rolling. Surface soil, clay loam, 44 centimeters deep; under cultivation for thirty-five to forty years.

Laboratory No. 4.—Tanauan Citrus Station, block C. Planted to mandarin trees, bore heavily in 1919–1920, since then light crops were obtained. No fertilization. Slightly rolling. Surface soil, 34 centimeters deep; under cultivation for thirty-five to forty years.

Laboratory No. 7.—Tanauan Citrus Station, variety test plot. Planted to mandarin trees 1 to 3 years old, formerly the plot was occupied by productive trees. No fertilization. Slightly rolling. Surface soil, clay loam, 56 centimeters deep; under cultivation for thirty-five to forty years.

Laboratory No. 8.—Tanauan Citrus Station, miscellaneous plot. Planted to mandarin trees from 1 to 3 years old. No fertilization. Slightly rolling. Surface soil, clay loam, 35 centimeters deep; under cultivation for thirty-five to forty years.

MOUNTAIN PROVINCE, LUZON

Laboratory No. 18.—Bontoc, Calottit, semitemperate substation, field A, No. 1. Planted to nursery citrus plants. No fertilization. Rolling. Surface soil, clay, 4 centimeters deep. Under cultivation for four years.

Laboratory No. 19.—Bontoc, Calottit, semitemperate substation, field A, No. 2. Planted to coffee seedlings. No fertilization. Rolling. Surface soil, clay with rock, 45 centimeters deep. Under cultivation for four years.

Laboratory No. 20.—Bontoc, Calottit, semitemperate substation, field A, No. 3. Planted to nursery plants, mango stocks. No fertilization. Rolling. Surface soil, clay loam, 60 centimeters deep. Rocky subsoil.

Laboratory No. 21.—Bontoc, Calottit, semitemperate substation, field B, No. 1. Planted to hubar clover. No fertilization. Rolling. Surface soil, clay loam, rocky subsoil, about 30 centimeters deep.

Laboratory No. 22.—Bontoc, Calottit, semitemperate substation, field B, No. 2. Planted to loquats and peanuts. Fertilized with green manure. Rolling. Surface soil, clay loam, 90 centimeters deep, subsoil not so rocky.

Laboratory No. 23.—Bontoc, Calottit, semitemperate substation, field B, No. 3. Planted to early Crawford. Fertilized with green manure (leguminous plant). Rolling. Surface soil, clay loam, 60 centimeters deep. Subsoil not so rocky.

Laboratory No. 24.—Bontoc, Calottit, semitemperate substation, field B, No. 4. Planted to fruit trees, apples. Cowpeas used as green manure. Rolling. Surface soil, clay loam, 90 centimeters deep, subsoil rocky.

Laboratory No. 25.—Bontoc, Calottit, semitemperate substation, field B, No. 5. Planted to apples and peanuts. Leguminous green manure plowed under. Rolling. Surface soil, clay loam, 90 centimeters deep, rocky substratum.

Laboratory No. 26.—Bontoc, Calottit, semitemperate substation, field C, No. 1. Planted to fruit trees. No fertilization. Rolling. Surface soil, clay loam, 60 centimeters deep, substratum rock with clay. Newly cultivated.

Laboratory No. 27.—Bontoc, Calottit, semitemperate substation, field C, No. 2. Planted to fruit trees. No fertilization. Rolling. Surface soil, clay loam, 75 centimeters deep, substratum rock with clay. Newly cultivated.

Laboratory No. 28.—Bontoc, Calottit, semitemperate substation, field C, No. 3. Planted to fruit trees. No fertilization. Rolling. Surface soil, clay loam, 60 centimeters deep, substratum rocky. Newly cultivated.

Laboratory No. 29.—Bontoc, Calottit, semitemperate substation, field C, No. 4. Planted to fruit trees. No fertilization. Rolling. Surface soil, clay loam, 60 centimeters deep, rocky substratum.

Laboratory No. 30.—Bontoc, Calottit, semitemperate substation, field C, No. 5. Planted to fruit trees. No fertilization. Rolling. Surface soil, clay loam, 30 centimeters, with rocky substratum.

OCCIDENTAL NEGROS PROVINCE, NEGROS

Laboratory No. 49.—Bacolod, Hacienda Helvetia, owned by Jose de la Rama, fields 47, 48, 49, and 50. Planted to sugar cane. Fertilized with Sumatra and Corona No. 1, 500 kilograms per hectare. Plain. Surface soil, clay loam, very shallow. Drainage fair.

Laboratory No. 50.—Bacolod, Hacienda Helvetia, owned by Jose de la Rama, fields 58, 61, and 62. Planted to sugar cane. Fertilized with Sumatra and Corona No. 1, 500 kilograms per hectare. Slightly rolling. Surface soil, clay loam, rather shallow. Efficient drainage.

Laboratory No. 51.—Bacolod, Hacienda Helvetia, owned by Jose de la Rama, field 84. Planted to sugar cane. Fertilized with Sumatra and Corona No. 1. Thin surface soil, clayey in nature. Under cultivation for several years.

Laboratory No. 52.—Bacolod, Hacienda Socorro, owned by G. Villanueva, field 8. Planted to sugar cane, first year ratoon. Fertilized with Big Crop at the rate of 500 kilograms per hectare. Slightly rolling. Surface soil, locally called *abo-abo* soil.

Laboratory No. 57.—Bacolod, Hacienda Rosario, owned by Rafael Alunan. Planted to sugar cane. Fertilized with complete fertilizer at the rate of 500 kilograms per hectare. Slightly rolling. Deep surface soil, clay loam. Good drainage.

Laboratory No. 58.—Bacolod, Hacienda Madalagan, owned by S. Abasquez. Field prepared for cane. No fertilization. Plain. Deep surface soil, sandy clay.

Laboratory No. 67.—Isabela, Hacienda Constancia. Sugar cane fertilized with Big Crop and ammonium sulphate. Brown silt loam, surface soil about 30 centimeters deep. Plain.

Laboratory No. 68.—Isabela, Hacienda San Bonifacio. Sugar cane fertilized with Big Crop and ammonium sulphate. Surface soil about 20 centimeters deep. Plain.

Laboratory No. 69.—Isabela, Hacienda Alicante. Cane fertilized with Big Crop. Surface soil, clay, about 25 centimeters deep. Plain. Under cultivation for several years.

Laboratory No. 70.—Isabela, Hacienda Sto. Domingo. Cane fertilized with Big Crop and ammonium sulphate. Surface soil, clay, about 24 centimeters deep. Under cultivation for several years.

Laboratory No. 71.—Isabela, Hacienda Pasanghilan. Formerly rice land to be planted to sugar cane. No fertilization. Heavy clay, about 40 centimeters deep.

Laboratory No. 59.—La Carlota, Hacienda Caiñaman, owned by G. Villanueva. Planted to sugar cane. No fertilization. Sandy clay over clay and gravel. Under cultivation for several years.

Laboratory No. 60.—La Carlota, Hacienda Camanog, owned by G. Villanueva, field 1. Planted to sugar cane. No fertilization. Last year's yield was 4 tons per hectare. Surface soil rather thin, brown silt loam over red clay. Plain.

Laboratory No. 62.—La Carlota, Hacienda Camanog, owned by G. Villanueva, field 2. Cane with no fertilization. Last crop was 4 tons to the hectare. Surface soil, brown clay over gravel. Plain. Good drainage.

Laboratory No. 61.—La Carlota, Hacienda La Paz. Cane with no fertilization. Four tons to the hectare in last year's crop. Thin surface soil, clay. Plain. Drainage not so efficient.

Laboratory No. 84.—La Carlota Experiment Station, field A, No. 1. Planted to sugar cane; in previous years it was in corn. Treated with lime shell at the rate of 1,000 kilograms to the hectare. Clay loam, surface soil. Plain. Good drainage.

Laboratory No. 85.—La Carlota Experiment Station, field A, No. 2. Control plot, with cane, but previously in corn. No fertilization. Clay loam. Plain. Good drainage.

Laboratory No. 86.—La Carlota Experiment Station, fields C and D, No. 3. Planted to sugar cane, previously in corn. Fertilized with Alco at the rate of 500 kilograms per hectare. Clay loam. Plain. Good drainage.

Laboratory No. 87.—La Carlota Experiment Station, fields E, F, G, and I, No. 4. Planted to cane. No fertilization. Clay loam soil, about 37 centimeters deep. Plain. Good drainage.

Laboratory No. 88.—La Carlota Experiment Station, field H-1, No. 5. Planted to sugar cane and fertilized with mungo as green manure. Clay loam surface soil, about 33.2 centimeters deep. Plain. Efficient drainage.

Laboratory No. 89.—La Carlota Experiment Station, field H-2, No. 6. Planted to sugar cane and fertilized with cowpeas as green manure. Clay loam, surface soil, about 27.3 centimeters deep. Plain. Efficient drainage.

Laboratory No. 90.—La Carlota Experiment Station, field H-3, No. 7, control plot. Planted to sugar cane, with no fertilization. Clay loam surface soil, 23.3 centimeters deep. Plain. Efficient drainage.

Laboratory No. 91.—La Carlota Experiment Station, fields J and L, No. 8. Planted to sugar cane, with no fertilization. Clay loam surface soil, 26.4 centimeters deep. Plain.

Laboratory No. 92.—La Carlota Experiment Station, field T, No. 9. Planted to sugar cane, with no fertilization. Clay loam surface soil, 25.2 centimeters deep. Slightly rolling.

Laboratory No. 93.—La Carlota Experiment Station, field R-1, No. 10. Planted to sugar cane, with no fertilization. Clay loam surface soil, 24.8 centimeters deep. Plain.

Laboratory No. 94.—La Carlota Experiment Station, field R-2, No. 11. Sugar cane, with ammonium sulphate at the rate of 40 kilograms nitrogen per hectare. Surface soil clay loam, 25.2 centimeters deep.

Laboratory No. 95.—La Carlota Experiment Station, field R-3, No. 12. Cane, fertilized with Anaconda fertilizer at the rate of 25 kilograms phosphoric acid per hectare. Clay loam soil. Plain.

Laboratory No. 96.—La Carlota Experiment Station, field R-4, No. 13. Cane, fertilized with sulphate of potash at the rate of 30 kilograms potassium oxide per hectare. Clay loam soil. Plain.

Laboratory No. 97.—La Carlota Experiment Station, field R-5, No. 14. Cane, fertilized with slaked lime at the rate of 500 kilograms per hectare. Clay loam soil. Plain.

Laboratory No. 98.—La Carlota Experiment Station, field R-6, No. 15. Cane, fertilized with ammonium sulphate and Anaconda fertilizer at the rate of 40 kilograms of nitrogen and 25 kilograms of phosphoric acid per hectare. Clay loam soil. Plain.

Laboratory No. 99.—La Carlota Experiment Station, field R-7, No. 16. Cane, fertilized with ammonium sulphate and sulphate of potash at the rate of 40 kilograms nitrogen and 30 kilograms of potassium oxide per hectare. Clay loam soil, 23.2 centimeters deep.

Laboratory No. 100.—La Carlota Experiment Station, field R-8, No. 17. Cane, fertilized with ammonium sulphate and sulphate of potash at the rate of 25 kilograms nitrogen and 30 kilograms potassium oxide per hectare. Clay loam soil, 22.4 centimeters deep.

Laboratory No. 101.—La Carlota Experiment Station, field R-9, No. 18. Cane, fertilized with ammonium sulphate, Anaconda, and sulphate of potash at the rate of 40 kilograms nitrogen, 25 kilograms phosphorus pentoxide, and 30 kilograms potassium oxide per hectare. Clay loam soil, 24.2 centimeters deep.

Laboratory No. 102.—La Carlota Experiment Station, field R-10, No. 19. Cane, fertilized with ammonium sulphate, Anaconda, sulphate of potash and lime at the rate of 40 kilograms nitrogen, 25 kilograms phosphorus pentoxide, 30 kilograms potassium oxide, and 500 kilograms lime per hectare. Clay loam soil, 27.2 centimeters deep.

Laboratory No. 103.—La Carlota Experiment Station, field N, No. 20. Planted to corn previously, sugar cane since 1923. No fertilization. Surface soil, clay loam, 19.8 centimeters deep.

Laboratory No. 104.—La Carlota Experiment Station, field No. 21. Planted to abacá with no fertilization. Clay loam, 26.6 centimeters deep. Plain.

Laboratory No. 105.—La Carlota Experiment Station, field No. 22. Planted to coffee, with fertilization. Clay loam soil on rocky subsoil. Surface soil 14.4 centimeters deep. Rolling.

Laboratory No. 106.—La Carlota Experiment Station, field No. 23. Planted to coffee, with no fertilization. Clay loam soil on rocky subsoil. Surface soil 12.4 centimeters deep. Rolling.

Laboratory No. 64.—La Castellana, Hacienda Manzanalao. Planted to sugar cane, first year. No fertilization. Surface soil about 80 centimeters deep. Slightly rolling.

Laboratory No. 63.—Maa Central, Hacienda Pangulayan. Planted to sugar cane, fertilized with Corona, Big Crop, and ammonium sulphate. Slightly rolling surface soil about 15 to 20 centimeters deep, impervious subsoil. Last year's yield 44 piculs.

Laboratory No. 65.—Maa Central, Hacienda Ibong. Planted to cane. Fertilized with Big Crop and ammonium sulphate. Surface soil about 20 centimeters deep. Last year's yield 80 piculs per hectare.

Laboratory No. 66.—Maa Central, Hacienda Zaragoza, Ledesma. Planted to sugar cane. Fertilized with ammonium sulphate and Big Crop. Surface soil about 20 centimeters deep, clay loam. Yield per hectare was 105 piculs.

Laboratory No. 72.—Maa Central, Hacienda Actividad. Planted to sugar cane. Fertilized with ammonium sulphate and Big Crop. Normal yield. Plain. Surface soil, 60 centimeters deep. Dark brown clay over sand.

Laboratory No. 73.—Maa Central, Hacienda Bulubugnay. Sugar cane, fertilized with Corona and Big Crop. Surface soil about 20 centimeters clay loam over impervious clay.

Laboratory No. 74.—Maa Central, Hacienda Bantolinao. Sugar cane, fertilized with ammonium sulphate and Big Crop, plain surface soil about 20 centimeters deep, red clay.

Laboratory No. 75.—Maa Central, Hacienda Zaragoza Montilla. Sugar cane, fertilized with ammonium sulphate and Big Crop. Gave normal yield. Slightly rolling surface soil 20 centimeters deep.

Laboratory No. 76.—Maa Central, Hacienda Buenavista. Sugar cane, fertilized with Big Crop and ammonium sulphate. Gave normal yield. Surface soil clay loam over impervious clay. Drainage rather poor.

Laboratory No. 77.—Maa Central, Hacienda Cubacubag. Sugar cane, fertilized with Big Crop and Corona No. 1. Normal yield was obtained. Surface soil clay loam over clay and gravel.

Laboratory No. 78.—Maa Central, Hacienda Yding. Planted to sugar cane. No fertilizer applied. Surface soil about 20 centimeters deep. Slightly rolling.

Laboratory No. 79.—Maa Central, Hacienda Progreso. Sugar cane, fertilized with Corona No. 1, Big Crop, and ammonium sulphate. Surface soil clay loam over clay and gravel.

Laboratory No. 80.—Maa Central, Hacienda Bonifacio and Progreso. Sugar cane; fertilized with Corona No. 1, Big Crop, and ammonium sulphate. Normal yield was obtained. Surface soil clay loam about 30 centimeters deep. Slightly rolling.

Laboratory No. 81.—Maa Central, Hacienda Begonia. Cane, fertilized with Big Crop and ammonium sulphate. Gave normal yield. Slightly rolling. Surface soil about 30 centimeters deep.

Laboratory No. 82.—Maa Central, Hacienda Maragandang. Cane, fertilized with ammonium sulphate, Big Crop, and Corona. Normal yield. Clay loam over clay and gravel.

Laboratory No. 56.—Manapla, Hacienda Maja. Being prepared for cane, planted to cane previously. Not fertilized. Deep soil, red clay. Fairly good drainage.

Laboratory No. 53.—Philippine Hawaiian, Hacienda Basag, owned by C. Locsin, lime-experiment plot, control. Planted to cane, first-year ratoon. Fertilized with 500 kilograms ammonium sulphate per hectare. Plain. Surface soil clay loam about 35 centimeters deep. Fairly good drainage. Yield 88 piculs of sugar per hectare.

Laboratory No. 54.—Philippine Hawaiian, Hacienda Basag, owned by C. Locsin, lime-experiment plot, with lime. Planted to cane, first-year ratoon. Treated with lime and fertilized with 500 kilograms ammonium sulphate. Surface soil clay loam 35 centimeters deep. Plain. Good drainage.

Laboratory No. 55.—Hacienda Badyong owned by V. Mappa, virgin soil. Uncultivated for several years. Deep surface soil, locally called abo-abo. Plain.

PANGASINAN PROVINCE, LUZON

Laboratory No. 9.—Rosales Rice Experiment Station, No. 1, variety test. Planted to lowland rice. Mungo and cowpeas were previously grown. No fertilization. Plain. Surface soil silt loam 30 centimeters deep. Good drainage. Under cultivation for several years.

Laboratory No. 10.—Rosales Rice Experiment Station, No. 2, propagation plot. Planted to lowland rice, previously grown to corn, mungo, and cowpeas. No fertilization. Plain. Surface soil silt loam 30 centimeters deep. Good drainage. Under cultivation for several years.

Laboratory No. 11.—Rosales Rice Experiment Station, No. 3, fertilizer-test plot. Planted to lowland rice. Mungo and cowpeas used as green manure. Fertilizer was not yet applied. Plain. Surface soil silt loam 30 centimeters deep. Good drainage. Under cultivation for several years.

Laboratory No. 12.—Rosales Rice Experiment Station, No. 4, dry-season plot. Planted to upland rice. Two crops are raised a year. No fertilization. Plain. Surface soil, silt loam, over 30 centimeters deep. Excellent drainage. Under cultivation for several years.

Laboratory No. 13.—Rosales Rice Experiment Station, No. 5, rotation plot. Rice rotated with sweet potatoes, gabis, and napier grass. No fertilization. Surface soil silt loam 30 centimeters deep. Plain. Drainage good.

RIZAL PROVINCE, LUZON

Laboratory No. 14.—Alabang Rice Station, No. A, paddy field. Planted to lowland rice. No fertilization. Plain. Surface soil clay 15 centimeters. Poor drainage.

Laboratory No. 16.—Alabang Rice Station, No. B-1, extension field. Planted to lowland rice. No fertilization. Surface soil clay 14 centimeters deep. Poor drainage.

Laboratory No. 17.—Alabang Rice Station, field C. Planted to sugar cane. No fertilization. Plain. Surface soil clay 29 centimeters deep. Poor drainage.

DISCUSSION OF TABLES 1, 2, AND 3

TABLE 1

Table 1 gives the nitrification results of all the soils analyzed. The first column gives the laboratory number of each soil sample, and the rest of the table is divided into series and each series is discussed separately. All the figures in this table are the results of duplicate tests, and were reported only when the difference between two determinations was not higher than 0.5 milligram of nitrate nitrogen.

Series I.—The figures in the first column indicate the quantity of nitrate nitrogen produced in 100 grams of each sample of untreated soil after it had been incubated under optimum conditions of moisture and temperature. The figures in the second column indicate the percentage of nitrogen converted into nitrate in each sample. The object in carrying out the experiment under this series was to find out how efficient certain soils are in converting their own nitrogen into nitrate, given proper aëration and optimum conditions of moisture and temperature.

Series II.—The experiment under this series consisted in adding ammonium sulphate to each sample of soil. The object was to get some idea as to how much of the nitrogen of ammonium sulphate could be converted into nitrate after a short period of incubation, but particularly to find out whether or not it would be profitable to apply ammonium sulphate to some of the soils under study. The effect of the treatment on the original reaction of the soil was also noted. The first column of the series presents the amount in milligrams of nitrate nitrogen produced per culture, and the second column the percentage of the added nitrogen nitrified.

Series III.—In this series, each soil sample was treated with ammonium sulphate and calcium carbonate. The amount of calcium carbonate was computed on the basis of the theoretical amounts of nitric and sulphuric acids formed from the decomposition of ammonium sulphate during the process of nitrification according to Waksman.⁽²⁹⁾ The original reaction of the soil was taken into account in the application of calcium carbonate. The purpose of the experiment was to learn the stimulating effect of lime in the form of calcium carbonate upon the nitrifying power of the soil, and also the maximum speed of nitrification from the easily nitrifiable source of nitrogen.

Series IV.—Dried blood has been used by most investigators in running nitrification tests; but, owing to lack of supply of this material in the laboratory where this work was conducted, copra cake was used in its place. Copra cake is a by-product of coconut-oil extraction. If the oil is extracted by hydraulic pressure, the oil content of the cake is from 7 to 8 per cent; in factories where other methods of extraction are used, the oil content is 12 to 15 per cent. The nitrogen content of copra cake is, on the average, about 3 per cent. Copra cake was used instead of other nitrogenous materials, because it is a home product and is easily obtained by the farmer. The purpose in treating the several soils under investigation with this material was to find out how much of its nitrogen content could be converted into available form under normal conditions, and also to ascertain whether the nitrate produced would be sufficient for the immediate need of heavy feeder crops such as sugar cane and corn.

Series V.—Each soil sample was treated with both copra cake and calcium carbonate. In every case calcium carbonate was applied in an amount slightly in excess of what the soil reaction would call for. With the application of calcium carbonate it was expected to increase the nitrifiability of copra cake and to get its full value as a nitrogenous fertilizer.

DISCUSSION OF RESULTS, TABLE 1

Batangas Province, soils grown to citrus trees.—The results of nitrification tests on citrus soils show a direct correlation between high nitrifying power and high productivity. In the case of ammonium sulphate treatment, samples 5 and 6, which were obtained from the most productive orchards, nitrified 42.30 per cent and 32.56 per cent, respectively, of the nitrogen added, whereas samples 1, 2, 3, and 4, from the unproductive orchards, nitrified only 15.77 per cent, 26.66 per cent, 21.28 per cent, and 20.77 per cent, respectively. This greater nitrification power of samples 5 and 6 as compared with that of other samples (except 4) was also observed in the case of copra cake. Samples 5 and 6 nitrified 41.66 per cent and 26.14 per cent, respectively, of the nitrogen of the copra cake, and sample 4, 32.75 per cent, whereas samples 1, 2, and 3 nitrified much smaller portions. Physically, however, the make-up of soils 4 and 6 is approximately the same, and chemically both soils contained the same amount of acid, which was equivalent to 120

TABLE 1.—Nitrification results.

TANAUAN CITRUS STATION AND NEIGHBORING ORCHARDS, BATANGAS PROVINCE

Soil No.	Series I, blank incubated.		Series II, ammonium sulphate.		Series III, ammonium sulphate and calcium carbonate.		Series IV, copra cake.		Series V, copra cake and calcium carbonate.	
	Nitrate nitrogen, per 100 grams culture.	Original nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.
	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.
1.....	3.60	3.00	5.50	15.77	31.00	79.48	3.90	11.20	14.90	42.81
2.....	3.10	2.58	10.40	26.66	26.90	69.97	4.90	14.08	7.50	21.55
3.....	2.50	2.35	8.30	21.28	22.30	63.68	6.40	18.39	16.10	46.26
4.....	1.00	.92	8.10	20.77	32.60	83.68	11.40	32.75	16.60	47.70
5.....	4.00	2.58	16.50	42.30	34.70	88.97	14.50	41.66	13.60	39.08
6.....	2.50	2.10	12.70	32.56	28.50	73.07	9.10	26.14	17.50	48.81
7.....	2.10	1.85	12.40	31.79	28.20	72.28	9.70	27.87	16.90	48.56
8.....	1.60	1.50	10.40	26.66	26.40	65.12	6.70	19.25	18.40	52.87

ROSALES RICE STATION, PANGASINAN PROVINCE

9.....	5.00	4.76	17.40	47.18	25.90	66.41	11.60	30.46	14.30	41.09
10.....	5.70	5.09	26.90	66.43	33.90	86.92	16.80	48.24	16.10	46.26
11.....	6.50	6.07	15.50	39.58	30.40	77.94	9.30	26.74	9.70	25.00
12.....	9.30	6.37	21.20	54.35	29.70	76.92	7.80	22.35	2.70	7.75
13.....	4.70	Trace	21.10	54.10	32.70	83.84	9.70	28.90	5.50	12.92

ALABANG RICE STATION, RIZAL PROVINCE

14.....	5.25	5.70	3.50	8.75	4.75	11.87	1.50	3.99	6.50	17.30
16.....	9.25	9.43	0.75	1.87	7.50	18.75	-----	-----	0.75	2.18
17.....	9.00	8.90	1.00	2.50	26.00	65.00	3.00	7.38	7.00	18.63

CALOTTIT STATION, BONTOC, MOUNTAIN PROVINCE

18.....	1.75	1.92	0.75	1.87	24.75	61.87	4.50	11.98	10.75	28.62
19.....	3.00	3.08	0.75	1.87	2.75	6.87	-----	-----	-----	-----
20.....	3.00	2.56	6.50	16.25	25.75	64.37	6.25	16.62	15.25	40.60
21.....	7.50	4.28	8.50	21.25	13.00	32.50	2.50	6.65	9.75	25.95
22.....	5.75	4.32	1.50	-----	17.50	43.75	3.00	-----	4.50	11.99
23.....	4.50	3.88	0.50	1.25	35.20	88.00	6.75	17.97	13.75	38.94
24.....	6.25	5.34	4.50	11.25	35.95	89.87	4.25	11.31	6.50	17.30
25.....	4.50	4.15	4.25	10.37	31.25	78.15	0.75	1.99	14.00	37.27
26.....	7.00	9.07	6.00	15.00	32.25	80.62	3.25	8.68	14.50	38.65
27.....	3.75	5.34	7.25	18.12	31.75	79.37	5.00	13.31	7.50	19.70
28.....	4.00	4.65	2.00	5.00	22.50	56.25	4.25	11.31	14.50	38.60
29.....	7.00	7.95	2.25	-----	38.00	95.00	0.75	-----	5.00	-----
30.....	4.50	5.33	2.25	5.76	13.50	33.75	0.25	0.66	1.50	3.99

TABLE 1.—Nitrification results—Continued.

LAMAQ STATION, BATAAN PROVINCE

Soil No.	Series I, blank incubated.		Series II, ammonium sulphate.		Series III, ammonium sulphate and calcium carbonate.		Series IV, copra cake.		Series V, copra cake and calcium carbonate.	
	Nitrate nitrogen per 100 grams culture.	Original nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.
	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.
31-----	5.25	6.56	10.50	26.25	10.50	26.25	16.50	43.92	-----	-----
32-----	5.25	5.09	9.75	24.37	27.50	68.75	14.50	38.59	19.75	62.58
33-----	4.50	9.37	16.25	46.25	37.25	92.55	15.50	41.26	17.50	46.59
34-----	11.25	15.20	11.50	28.75	30.00	75.62	2.00	5.99	14.00	37.96
35-----	Trace	-----	13.75	34.37	39.25	98.12	17.50	46.59	17.75	47.25
36-----	5.50	13.75	5.50	14.10	7.75	19.37	13.75	36.60	16.50	43.92
37-----	9.75	12.50	6.00	15.33	8.00	20.00	7.25	19.30	13.25	35.30
38-----	4.75	7.20	11.00	28.12	34.75	85.62	15.00	39.93	15.00	39.93
39-----	4.25	4.88	14.00	35.00	33.25	83.12	17.25	45.92	20.75	55.24
40-----	4.25	6.53	4.75	11.87	27.00	60.75	7.25	19.30	6.50	17.30
41-----	4.50	5.00	9.75	24.37	36.50	91.25	11.75	31.28	18.00	47.92
44-----	4.50	5.76	9.50	23.75	31.25	78.12	11.75	31.28	16.75	47.47
45-----	4.25	4.72	7.00	17.50	37.25	93.12	12.50	33.28	27.00	71.83
48-----	6.00	7.79	5.50	14.10	21.75	54.37	5.50	14.90	7.75	20.60

SUGAR-CANE PLANTATIONS, OCCIDENTAL NEGROS PROVINCE

49-----	4.75	6.41	2.25	5.76	15.75	40.38	8.25	21.99	17.75	47.25
50-----	1.87	2.96	1.88	4.80	4.36	11.21	8.41	9.11	10.61	29.61
51-----	5.00	6.11	5.50	14.10	24.25	61.17	10.00	26.62	15.00	39.93
52-----	Trace	-----	2.50	6.41	5.75	14.74	2.50	6.66	2.50	6.65
53-----	2.50	1.47	10.50	27.02	24.00	61.53	11.25	29.68	18.12	48.27
54-----	3.00	2.97	6.87	16.34	-----	-----	18.00	22.35	21.50	51.24
55-----	4.37	0.89	0.88	2.25	2.88	7.30	0.88	2.25	8.63	23.67
56-----	2.62	2.54	1.87	-----	7.13	18.28	2.25	-----	6.25	16.70
57-----	4.62	4.01	5.38	13.73	37.75	94.10	16.25	43.23	26.88	71.54
58-----	5.00	6.25	2.50	6.41	35.25	90.38	14.00	35.93	13.00	34.64
59-----	8.37	8.04	14.50	37.17	34.13	87.50	12.50	30.61	13.38	35.62
60-----	7.62	6.45	16.55	42.18	36.50	93.59	16.88	44.96	22.00	58.57
61-----	2.50	1.90	6.87	17.61	29.00	74.35	7.50	21.27	10.50	34.61
62-----	4.50	3.78	13.31	31.73	34.75	89.10	13.25	35.27	16.67	44.26
63-----	3.75	2.55	2.50	6.43	3.25	8.33	2.00	5.32	-----	-----
64-----	6.50	3.00	3.75	22.43	36.75	94.23	4.50	11.98	17.50	46.59
65-----	4.50	3.48	0.25	0.64	17.25	42.30	4.25	11.31	11.75	31.28
66-----	Trace	-----	4.50	11.53	22.50	57.69	8.25	21.96	16.75	44.65
67-----	1.75	1.86	7.00	17.50	34.75	86.87	8.50	22.66	18.00	47.92
69-----	2.00	2.19	19.50	48.75	37.25	93.12	17.50	46.59	17.50	46.59
70-----	1.75	1.50	12.50	31.25	38.50	96.25	14.25	37.98	14.25	37.98
71-----	1.75	0.73	15.50	37.58	30.00	75.00	11.50	30.61	18.50	49.25
72-----	4.50	2.68	2.50	6.24	25.50	62.50	1.50	6.65	13.00	34.64
74-----	6.25	1.43	27.25	68.12	33.00	82.50	16.25	43.26	11.25	29.96
76-----	Trace	-----	12.25	30.62	35.50	88.75	15.50	41.26	27.00	71.88
77-----	3.25	2.87	-----	-----	36.00	90.00	1.75	4.65	5.75	15.30
78-----	Trace	-----	6.00	15.00	20.00	50.00	10.00	26.62	20.00	53.27
79-----	2.50	2.38	2.50	6.25	28.00	70.00	5.00	13.31	6.75	18.00
81-----	3.25	1.31	-----	-----	19.50	48.75	3.50	9.31	13.50	41.93

TABLE 1.—Nitrification results—Continued.

LA CARLOTA STATION, OCCIDENTAL NEGROS PROVINCE

Soil No.	Series I, blank incubated.		Series II, ammonium sulphate.		Series III, ammonium sulphate and calcium carbonate.		Series IV, copra cake.		Series V, copra cake and calcium carbonate.	
	Nitrate nitrogen, per 100 grams culture.	Original nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.	Nitrate nitrogen.	Added nitrogen nitrified.
	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.
84.....	2.60	1.09	9.00	16.41	11.80	30.23	2.70	7.84	6.90	18.89
85.....	1.00	0.45	15.00	38.46	30.70	73.72	11.90	39.94	18.80	53.19
86.....	1.10	0.55	12.50	32.05	29.90	76.66	13.80	38.50	19.50	55.17
87.....	2.20	1.27	13.00	33.33	32.10	79.23	12.70	35.74	18.80	54.64
88.....	1.70	1.01	16.50	42.30			13.60	36.20	16.40	48.27
89.....	2.00	1.54	18.40	47.18	36.00	92.30	14.00	40.00	21.60	60.17
90.....	3.00	1.87	14.50	37.07	29.70	76.15	3.80	11.04	4.00	11.54
91.....	2.50	1.03	17.50	44.87	27.50	70.51	3.50	10.11	5.50	15.98
92.....	2.10		16.70	42.82	35.40	85.69	7.30	18.31	4.80	13.95
93.....	4.30	1.50	16.30	41.79	30.80	78.97	5.20	15.11	6.10	17.73
94.....	2.60	0.92	22.00	56.41	33.40	85.64	15.80	42.09	16.70	43.83
95.....	3.00	0.87	22.80	58.46	33.20	82.66	16.00	46.50	16.50	47.96
96.....	3.80	1.00	22.00	55.12	33.40	85.64	14.30	36.38	15.40	41.00
97.....	3.10	0.93	19.70	50.51	33.60	86.41	14.40	38.33	14.80	39.40
98.....	2.50	0.84	19.40	49.74	33.90	86.92	16.00	42.59	17.00	45.26
99.....	3.10	0.91	23.50	58.71	33.00	84.61	13.40	35.68	14.90	40.20
100.....	3.40	0.99	15.30	39.31	31.40	80.25	14.10	36.12	15.70	41.79
101.....	2.20	0.55	22.40	57.43	34.80	89.23	15.50	41.00	17.30	46.05
102.....	4.60	0.14	15.10	38.71	29.40	75.38	13.20	35.17	13.00	34.62
103.....	3.20	1.19	21.65	55.51	34.30	87.94	12.40	35.04	14.20	37.80
104.....	3.50	1.52	14.20	36.66	31.40	80.00	13.70	37.00	16.10	42.86
105.....	5.00	1.91	15.00	38.46	32.25	82.69	12.80	37.20	13.10	36.20
106.....	3.40	1.16	16.00	41.02	32.00	83.68	15.30	40.75	12.90	37.50

kilograms calcium carbonate per hectare. Presumably the buffer actions of these two soils, with regard to acid and alkali, are the controlling factors, which may account for the difference in results. According to Waksman's (29) findings, ammonium sulphate could be applied to a well-buffered acid soil without the activities of the nitrifying bacteria being hindered; but, in a poorly buffered acid soil, the effect of the acid practically stops nitrification. In this connection the views of Ruprecht and Morse (23) with regard to the application of ammonium sulphate are worthy of consideration. They claimed that the injurious effect was due to the formation of the sulphates of magnesium, iron, and aluminium, rather than to the acids produced. When ammonium sulphate and copra cake were

applied with calcium carbonate, the nitrifying powers of the soils were accelerated to a much higher degree. Undoubtedly these soils had a sufficient number of nitrifying bacteria and could be made to nitrify under normal conditions.

Rosales Rice Station soils.—One of the most striking points presented in the table is the high nitrifiability of ammonium sulphate as compared to that of copra cake in soils of this section of Pangasinan Province. When ammonium sulphate and copra cake were applied separately, 52.32 per cent of the nitrogen of the former was nitrified, whereas of the latter only 31.33 per cent on an average was nitrified. When the same materials were applied with calcium carbonate, an increase in nitrate production was observed in the case of ammonium sulphate, but there was a decrease in the case of copra cake. In general, all the soils tested nitrified very efficiently the original nitrogen content and the nitrogen of the materials used, such as ammonium sulphate and copra cake. When ammonium sulphate was applied in combination with calcium carbonate, the nitrate production increased from 52.32 per cent to 78.40 per cent; but when copra cake was used with calcium carbonate the nitrate production decreased from 31.33 per cent to 26.60 per cent. Since these soils were only slightly acidic in reaction, the decrease in nitrate production was due to the lime added, which affected the activities of the organisms concerned in the process of nitrification. This effect, however, was not noticed with ammonium sulphate, because sulphuric acid was being produced during the decomposition of the sulphate, which undoubtedly sufficed to counteract the alkalinity of the calcium carbonate.

Alabang Rice Station soils.—The nitrification results obtained from the study of the soils in this station were very low, the highest for ammonium sulphate being 8.75 per cent, and the lowest 1.89 per cent. For copra cake the highest was 7.38 per cent, and the lowest negative. Upon the addition of calcium carbonate to these materials, higher nitrification results were obtained. The physical analyses of these soils showed, without exception, a high content of clay. Seemingly, the physical effect derived from the application of lime stimulated the activities of the bacteria concerned in the process of nitrification. The poor physical properties of these soils, and this low content of nitrogen, phosphorous, potassium, and other, less-important elements may account for the low nitrifying power of these soils.

Calottit, Semitemperate Station soils.—The high nitrate production found in all of these soils after a period of incubation was due to the previous application of leguminous plants as green manure, and the addition of nitrogen, in the form of either ammonium sulphate or copra cake, did not increase their nitrifying power to a noticeable extent. When either ammonium sulphate or copra cake, alone, was applied to these soils only a small portion of their nitrogen content was nitrified; but, when each was applied in combination with calcium carbonate, a larger portion of their nitrogen was converted into nitrate. The principal reasons for the low nitrification of the nitrogen of the added materials were the presence at the time of sampling of sufficient quantities of nitrogenous materials the nitrogen of which is easily nitrified and, possibly, the insufficient numbers of nitrifying organisms present. From the physical standpoint, these soils offer the best conditions for optimum nitrification. Their percentage of clay is much lower than that of other soil separates.

Lamao Station soils.—The efficiency of the soils in this station in converting their own original nitrogen into available form was higher than that of any other soils tested. The nitrogen of both ammonium sulphate and copra cake, either applied alone or in combination with calcium carbonate, was highly nitrified. This result seems to indicate that these soils are capable of converting the nitrogen of any nitrogenous material into available form, thereby insuring a sufficient supply of nitrate for the maximum crop yield.

Soils from different plantations of Occidental Negros.—After a careful examination of the nitrification results of each of the individual fields, it was found that low nitrification was closely associated with the poor physical condition of the soil. For example, sample 56 was found, upon physical analysis, to contain 44.89 per cent clay, and the percentage of gravel was also high. When ammonium sulphate was applied alone to this soil, it was not nitrified after an incubation of twenty-eight days at 28° C. The same result was obtained in the case of copra cake. The addition of calcium carbonate to ammonium sulphate and copra cake induced nitrification to only a small degree. As this soil was also acid in reaction, the use of the calcium carbonate made possible the reduction of its active acids and improvement of its physical condition. However, the plantation soils tested were in general found to be efficient in convert-

ing the nitrogen of ammonium sulphate and copra cake into available form. Taking into consideration the methods of cultivation, the manner of application, and the kinds of fertilizers used, the results correlate with the average yields of the fields, judged from my observation on the stand of cane at the time of sampling.

La Carlota Experiment Station soils.—Of all the Negros soils tested, those from this station showed the highest nitrifying power in general; but the ability of these soils to nitrify their own original nitrogen was low. The probable reasons are the following: (a) These soils have been under intensive cultivation for a number of years and, by continued cropping, have become toxic to the nitrifying bacteria and (36, 21) been rendered inactive; (b) the nitrogenous materials which these soils contained were not easily broken up and the twenty-eight-day period of incubation allowed in the experiment was not sufficient to bring about their maximum decomposition; (21) and (c) corn was previously grown in those fields which, being a heavy feeder crop, depleted the soil of its essential elements.

TABLE 2

The figures in Table 2 give the average results of nitrification for each locality or district. The object in preparing this table was to show the degree of variation in the nitrifying power of the soils from different parts of the Islands. In spite of the heavy application of commercial fertilizers in the sugarcane districts of Occidental Negros, the ability of the soils in those districts to convert the original nitrogen into available form was less than that of the soils of other districts where different crops, such as rice, tobacco, coconut, abacá, citrus, etc., are grown. Nevertheless, the data show that the sugarcane districts are rather efficient in nitrifying ammonium sulphate and copra cake, and possibly other nitrogenous fertilizers.

Alabang soils show poor nitrifying power. The figure 8.01 under the column "per cent of original nitrogen nitrified" does not necessarily mean that these soils are highly efficient in converting their original nitrogen into available form. The result of the physical analysis indicates that these soils are very retentive, as they contain a high percentage of clay. Such soil characteristic is generally found to facilitate a high accumulation of ammonia. Consequently, when these soils were subjected to oxidation under favorable conditions, the ammonia already present was converted into nitrate. It was observed that dur-

TABLE 2.—Average nitrification for each district.

Location.	Series I, blank incu- bated.		Series II, ammonium sulphate.		Series III, ammonium sulphate and calcium car- bonate.		Series IV, copra cake.		Series V, copra cake and cal- cium carbonate.	
	Nitrate nitro- gen per 100 grams culture.	Original ni- trogen nitrif- ied.	Nitrate nitro- gen.	Added nitro- gen nitrified.	Nitrate nitro- gen.	Added nitro- gen nitrified.	Nitrate nitro- gen.	Added nitro- gen nitrified.	Nitrate nitro- gen.	Added nitro- gen nitrified.
	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.	mg.	P. ct.
1. Batangas Prov- ince, Tanauan Experiment Station and other citrus or- chards.....	2.55	2.11	10.16	25.97	28.82	74.50	8.32	23.66	15.18	43.45
2. Bataan Province, Lamiao Exper- iment Station....	5.23	8.05	9.62	21.67	27.28	67.64	12.00	31.97	16.19	43.37
3. Mountain Prov- ince, Bontoc, Ca- lottit Experi- ment Station....	4.82	4.76	3.93	7.86	24.73	62.27	8.70	10.04	10.50	26.96
4. Occidental Negros Province, La Carlota Exper- iment Station....	2.40	10.1	17.45	44.34	31.42	79.19	12.64	33.43	14.34	39.88
Plantations.....	3.93	3.19	6.95	20.30	26.76	63.92	9.24	23.69	13.59	39.35
5. Pangasinan Prov- ince, Rosales Rice Experi- ment Station....	6.24	5.57	20.42	52.32	30.52	78.40	11.04	31.33	9.26	26.60
6. Rizal Province, Alabang Rice Experiment Sta- tion.....	7.83	8.01	1.75	4.37	12.75	31.87	2.25	5.63	4.75	12.70

ing the process of nitrification, the formation of ammonia was slower than the oxidation of ammonia to nitrate. (21) Tanauan, Rosales, and Lamiao soils, in general, are rather efficient in their nitrifying power. The organisms concerned are made active by the addition of calcium carbonate. Of the soils of these three stations, the Lamiao soil shows highest efficiency in nitrifying its own original nitrogen content, the percentage being 8.05. Calottit, a semitemperate district of the Islands, has lower nitrifying power as compared with the nitrifying power of the soils from other places, except those from Alabang station. As Calottit is located in a district of higher elevation where the land is rolling in character, the fertility of its soils has been depleted to a considerable extent, due to the excessive washing from yearly

precipitation and, according to Waksman's⁽²⁷⁾ investigation, the numbers and activities of the soil organisms are in direct correlation with the fertility. This, as one factor, may account for the low nitrifying power of the soils from this district.

TABLE 3

Table 3 gives the average results of the nitrification tests for the Philippine, the Hawaiian, and the United States soils. The Philippine soils were subjected to similar laboratory conditions as were the soils of the other two countries. The difference, however, lies in one particular treatment, in which copra cake was used for the Philippine soils and dried blood for the Hawaiian and the United States soils. As far as the nitrifiability of these two materials was concerned very little difference had been observed. I assumed that such variation would not materially influence the comparison of the nitrifying power of the soils of the three countries.

The original nitrogen in the Philippine soils examined undergoes a greater degree of nitrification than does that in the Hawaiian soils, but very much less than that in the United States soils. When ammonium sulphate was added, the Philippine soils nitrified 25.26 per cent of the added nitrogen, while the United States soils nitrified 23.75 per cent. The Hawaiian soils nitrified almost twice as much as did either of the other two countries. In the case where the nitrogen added was derived from an organic source, the Philippine soils showed a lower nitrification power than did the Hawaiian, but approximately the same as the United States soils. It is logical, however, to expect that the difference in the activities of soil bacteria would occur between the Philippine and the United States soils, because the climatic conditions of these two countries are extremely different; but the difference was very pronounced between the Hawaiian and the Philippine soils, climatic conditions being similar in many respects, and therefore it must be assumed that factors other than climatic influence were responsible for this difference in results.

From my personal observation during my short stay in the Hawaiian Territory, traveling from one island to another, the soils are all of volcanic origin, highly loaded with the oxides of aluminium and iron; organic matter is not very abundant. In general the soils are porous, offering excellent aëration.⁽⁷⁾ After several qualitative tests in the fields with the solution of potassium sulphur cyanate⁽⁹⁾ most of the soils were found only

TABLE 3.—Average nitrifying power of some of the Philippine soils as compared to those of Hawaii and the United States.

Country.	Series I, blank incubated.		Series II, ammonium sulphate.		Series III, ammonium sulphate and calcium carbonate.		Series IV, copra cake.		Series V, copra cake and calcium carbonate.	
	Nitrate nitrogen per 100 grams culture.	Original nitrogen nitrified.	Nitrate nitrogen per 100 grams culture.	Added nitrogen nitrified.	Nitrate nitrogen per 100 grams culture.	Added nitrogen nitrified.	Nitrate nitrogen per 100 grams culture.	Added nitrogen nitrified.	Nitrate nitrogen per 100 grams culture.	Added nitrogen nitrified.
Philippine Islands..	mg. 4.72	P. ct. 4.55	mg. 7.75	P. ct. 25.26	mg. 26.04	P. ct. 65.39	mg. 8.45	P. ct. 22.82	mg. 11.79	P. ct. 33.18
Hawaiian Territory.	8.06	0.44	21.78	48.90	-----	-----	^a 12.82	^b 29.00	^b 21.29	^b 49.90
United States (every state and territory included)	15.96	14.62	11.23	23.75	-----	-----	^a 26.53	^a 19.65	-----	-----

^a Dried blood.^b Dried blood and calcium carbonate.

slightly acid. In practically all the places visited, drainage was found to be very efficient; hardly any stagnant water or water-logged soil was noticed. On the other hand, the greater number of Philippine soils are residual, containing a considerable amount of organic matter. The amount of iron and aluminium oxides is not so high as that found in Hawaiian soils. Most of the soils are clayey, underlain with an impervious stratum, giving unsatisfactory drainage. Judging from the above description, therefore, the Hawaiian soils offer the most favorable conditions for maximum nitrification.

In general, the nitrifying power of the Philippine soils tested for ammonium sulphate and copra cake, with or without the addition of calcium carbonate, is considerably lower than that of either the Hawaiian or the United States soils. However, the original nitrogen in the Philippine soils examined is more readily nitrified than is that in the soils from the two countries cited. This may be attributed to the following factors:

a. The physical make-up of Philippine soils is clayey in nature and underlain with an impervious stratum, which renders drainage and aëration unsatisfactory.

b. The prolonged and extreme drying of the soils which occurs during the summer months, reduces very materially the number and activities of the soil bacteria. In previous work,(1) I demonstrated conclusively the effect of prolonged desiccation, under laboratory conditions, upon the viability of legume bacteria, azotobacter, and *Bacillus radiobacter*.

c. Most of the Philippine soils are acidic in reaction.

It was noticed that in all normal soils, ammonium sulphate was nitrified sooner than was copra cake. This result, of course, is not surprising; it simply illustrates the number of stages taking place during the process of nitrification. In the conversion of ammonium sulphate into nitrate only the following three stages are concerned, and these stages are easily carried on without the expense of surplus energy: (a) The breaking down of ammonium and sulphate radicals into ammonium hydroxide and sulphuric acid; (b) the oxidation of ammonia to nitrite; and (c) the further oxidation of nitrite to nitrate. However, in the case of copra cake, at least four important steps are necessary, namely: (a) The breaking down of the organic matter from complex protein compounds into simpler ones; (b) the hydrolysis of nitrogen radical to ammonium hydroxide; (c) the oxidation of this ammonia to nitrite; and (d) the further oxidation of nitrite to nitrate.

The possible biological reactions concerned in the decomposition of ammonium sulphate and copra cake are:

Ammonium sulphate—

1. $(\text{NH}_4)_2\text{SO}_4 + 2\text{HOH} \rightarrow 2\text{NH}_4\text{OH} + \text{H}_2\text{SO}_4$.
2. $\text{NH}_4\text{OH} + 3\text{O} \rightarrow \text{HNO}_2 + 2\text{HOH}$.
3. $\text{HNO}_2 + \text{O} \rightarrow \text{HNO}_3$.

Copra cake—

1. Sample $\xrightarrow{\text{broken down by molds and cellulose bacteria.}}$ $\left\{ \begin{array}{l} \text{Simple protein} \\ \text{compounds contain-} \\ \text{ing nitrogen.} \end{array} \right.$
2. (Protein compounds) $\text{R}-\text{N} \xrightarrow{\text{hydrolyzed by ammonifiers}}$ $\text{R} \text{ and } \text{NH}_4\text{OH}^2$
3. $\text{NH}_4\text{OH} \xrightarrow[\text{Nitrosomonas}]{\text{oxidized by}}$ HNO_2 .
4. $\text{HNO}_2 \xrightarrow[\text{Nitrobacter}]{\text{oxidized by}}$ HNO_3 .

In almost all cases, the application of calcium carbonate increased the nitrifying power of the soils. The object of using

² It has been observed by Löhnis and Fred that the decomposition of organic matter to ammonia is a difficult one, and time is an important consideration in the rate of decomposition.

calcium carbonate is twofold; namely, to correct the physical condition of the sticky soils and to neutralize their acidity. From the practical point of view the latter is the more important and worthy of consideration. The use of calcium carbonate or lime with the idea of correcting the physical condition of the soil is prohibitive in agricultural practice, because a heavy application is required to give any appreciable effect. Since neutralizing acidity is the more important function of calcium carbonate, it should be used only when the soil is acid. Although Burgess⁽⁸⁾ noticed increase in nitrification from the use of lime carbonate in alkaline soil, he attributed this increase to the physical rather than to the chemical effect. It should be remembered also that during the process of nitrification a strong acid is liberated, making the reaction of the medium very unfavorable to the nitrifying organisms; and unless this acid is neutralized their activities stop, resulting in the delay of nitrification. Hence the presence of calcium carbonate in the soil is very necessary; it encourages nitrification and accelerates to the maximum the activities of the nitrifying bacteria.

SUMMARY

1. The results obtained in the treatment of citrus soils constitute conclusive evidence that nitrification is an index of crop production. Soils from productive orchards showed higher nitrifying power than did soils from unproductive orchards.

2. In general, soils devoted to sugar cane converted their original nitrogen into available form more slowly than did those devoted to rice, abacá, tobacco, coconut, citrus, etc. The average nitrogen for sugar-cane soils was 2.10 per cent, while for the other soils it was 5.70 per cent.

3. It was found that the physical property of the soil has an important bearing on its nitrifying power. Soils containing a high percentage of clay nitrified either the original or the added nitrogen very poorly.

4. The addition of calcium carbonate to most of the soils accelerated their nitrifying power, resulting in high production of nitrate; but its application to the Rosales soils proved injurious to the activities of the organisms concerned, and this may account for the low nitrifying power of those soils.

5. In general, ammonium sulphate was converted into nitrate faster than was copra cake. The changes which these materials undergo during the process of nitrification account for the difference in the rate of decomposition.

6. On the average, the nitrifying power of some of the Philippine soils was lower than that of the Hawaiian. Possibly the factors responsible for this difference are the physical and chemical effect of the soil properties, and also the effect of climatic conditions upon the activities of the soil organisms.

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FREE TOXIN IN THE BLOOD DURING THE COURSE OF TETANUS TOXÆMIA

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Considerable work has been done on the resorption of tetanus toxin with the particular object in view of learning whether the toxin is resorbed by way of the nerves or by way of the blood stream.

Gumprecht, Goldscheider, Zupnik,² and others claimed that in general tetanus the toxin is conveyed to the central nervous system by the blood stream; but Mayer and Ramson³ maintained that the toxin is transported by peripheral nerves.

Sawamura,⁴ experimenting on rabbits, proved that the form of tetanus depends on the amount of injected toxin and the region where the injection is made. If tetanus toxin is given directly into the muscle or subcutaneously where the skin is directly attached to the muscle, ascending tetanus will develop; but, if the toxin is given in a place where there is no muscle, descending tetanus will take place.

The present experimental study was undertaken as a supplement to a clinical test performed by one of us on a patient suffering from chronic tetanus.

A young boy, 11 years of age, developed rigidity of the muscles of the face, of the trunk, and of the extremities. There was no recent injury to which the infection could be attributed. The rigidity increased gradually. Diagnosis of tetany was made. A consulting physician was called after the malady had lasted for about two months and he requested a laboratory test, rather to convince the parents that the child was suffering from tetanus and not from tetany than to establish the diagnosis.

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² Kolle W., und H. Hetsch, *Die Experimentelle Bakteriologie und die Infektionskrankheiten*, VI. Auflage, 1: 559.

³ *Arch. f. Exp. Path. u. Pharm.* 49 (1903).

⁴ *Experimentelle Studien zur Pathogenese und Serumtherapie des Tetanus. Arbeiten a.d. Inst. z. Erforschung der Infektionskrankh. in Bern*, H. 4, Jena, G. Fischer (1909).

At the time one of us saw the patient there was a pronounced opisthotonus and typical "risus sardonicus" present, rigidity of all the muscles of the extremities and of the trunk muscles, the form and insertions of which were plainly visible upon inspection. None of the usual symptoms of tetany were present, so that the diagnosis of tetanus could be discarded. The patient presented a typical picture of tetanus with attacks of clonic spasm which occurred particularly at night and in which the diaphragm was evidently involved. Upon close questioning of the intelligent parents it was learned that the boy acquired a wound on his foot from a cut previous to his sickness and that he had had decayed teeth for some time. No evidence of the alleged wound could be found at the time of examination. There being no clue to the search for the focus of infection we suggested a test for free toxin. Five cubic centimeters of blood were withdrawn from a vein and placed in a test tube containing sterile sodium citrate. The entire amount of blood obtained by venepuncture was injected subcutaneously into a guinea pig to enable us to obtain the maximum effect.

The guinea pig, 300 grams body weight, developed typical symptoms of tetanus on the second day and died the fourth day after injection.

It is evident that the patient, several days before death, harbored in the circulating blood a considerable amount of free tetanus toxin after two and a half months of illness, since 5 cubic centimeters of his full blood contained at least one minimal lethal dose for a guinea pig of 300 grams body weight.

In the literature touching the present question a most interesting reference was found, that of Madsen,⁵ who demonstrated the presence of tetanus toxin in the blood of a horse five days before the onset of the symptoms.

The present experimental study was made for the purpose of demonstrating how far the presence of tetanus toxin can be demonstrated in the blood in the course of tetanus toxæmia.

Experiments with sterile toxin, which allowed an approximate quantitative estimation of the amount of toxin circulating in the blood of the experimental animal, were performed.

CHOICE OF EXPERIMENTAL ANIMALS

Guinea pigs were used as recipients of the tetanus toxin to be searched for in the blood stream. Guinea pigs were se-

⁵ Tetanusgift im Serum eines Pferdes, fünf Tage vor dem Ausbruch des Tetanus, *Zentralbl. f. Bakt.* 46 (1908).

lected for our work because of the type of tetanus they develop after subcutaneous injection of tetanus toxin in the supraumbilical region, and also for the reason that they are the most susceptible of small experimental animals. As Kolle^o states, the same amount of tetanus toxin is the minimal lethal dose for 1 gram of mouse, 12 grams of horse, 8 grams of guinea pig, 2 grams of goat, or 1.5 grams of rabbit. Mice were also used on account of their small size and susceptibility to tetanus toxin.

EXPERIMENTS ON ANIMALS WITH TETANUS TOXIN

A ripe, or well-settled, tetanus toxin was used, the original minimal lethal dose of which was 0.00007 cubic centimeter for a 300-gram guinea pig.

The general plan of the experiments was to inject decreasing doses of toxin into experimental animals and to withdraw the blood from them. The withdrawn blood was placed in a definite amount of sodium citrate and injected subcutaneously or intramuscularly into smaller animals, such as guinea pigs and mice. The details are evident from Table 1.

DISCUSSION

It is evident from Table 1 that the minimal lethal dose of the toxin in question was 0.0005 cubic centimeter, since a guinea pig of 300 grams body weight that received this dose subcutaneously died within four days after injection.

A guinea pig of 820 grams weight that received five hundred minimal lethal doses for a standard-weight guinea pig harbored, within twenty-four hours after the injection, in 2 cubic centimeters of its blood enough free tetanus toxin to kill a 235-gram guinea pig within forty-eight hours.

A guinea pig of standard weight (300 grams) which received two hundred minimal lethal doses harbored, within twenty-four hours after the injection, at least one minimal lethal dose for a guinea pig of its own weight in 0.5 cubic centimeter of its full blood. A 300-gram guinea pig that received forty minimal lethal doses for a guinea pig of standard weight harbored in 0.5 cubic centimeter of its blood, within twenty-four hours after the injection, at least one minimal lethal dose for a mouse of 15 grams body weight.

A standard-weight guinea pig, injected subcutaneously with ten minimal lethal doses for a guinea pig of standard weight,

^o Kolle und Hetsch, *Die Experimentelle Bakteriologie und die Infektionskrankheiten*, VI. Auflage, 1: 548-549.

TABLE 1.—*Showing the results of animal tests for free tetanus toxin in the circulating blood.*
 [s, slight stiffness; ss, distinct stiffness; ss, pronounced stiffness; D2, dead on the second day; D3, dead on the third day; D4, dead on the fourth day; D6, dead on the sixth day; D13, dead on the thirteenth day.]

Guinea pig No.	Body weight.	Subcutaneous amount.		Bled after infection.	Amount of blood injected into hind leg of guinea pig or mouse.			Days.							
		Minimal lethal doses.			Blood.	Animal.	Weight of animal.	1	2	3	4	5	6 or more.		
		cc.	Hrs.												
1	820	0.25	500	24	2	Guinea pig	235	s	D2						
2	300	0.3	600	24	0.5	do.	300	s	ss	ss	D4				
3	300	0.2	400	24	0.5	do.	300	s	ss	ss	ss	ss	D13		
4	300	0.1	200	24	0.5	do.	300	s	ss	D3					
5	300	0.03	60	24	0.5	Mouse.	15	ss	D3						
6	300	0.02	40	24	0.5	do.	15	ss	D3						
7	300	0.01	20	24	0.5	do.	15	ss	ss	ss	ss	ss	D6		
8	300	0.005	10	24	0.5	do.	15	s	s	ss	ss	ss	ss		
9	300	0.0025	5	24	0.5	do.	15								
10	300	0.001	2	24	0.25	do.	15								
11	300	0.0005	1	24		Guinea pig	300	s	s	ss	D4				
12	300	0.0001	0.5	24		do.	300	(s)	(s)	(s)	(s)	(s)	(s)	(s)	
13	300	0.00005	0.1	24		do.	300	(s)	(s)	(s)	(s)	(s)	(s)	(s)	

^a Lived with symptoms.

harbored, within twenty-four hours, in 0.5 cubic centimeter of its blood enough tetanus toxin to produce symptoms of tetanus in a mouse of 15 grams within twenty-four hours after the intramuscular injection into the mouse, while a guinea pig of standard weight that received subcutaneously five minimal lethal doses for a guinea pig of standard weight harbored enough tetanus toxin in 0.5 cubic centimeter of its blood, within twenty-four hours after the injection, to produce typical tetanus symptoms on the fourth day in a mouse of 15 grams body weight.

As can be seen from Table 1, it so happened that one 300-gram guinea pig received two hundred minimal lethal doses and, within twenty-four hours after the injection, 0.5 cubic centimeter of its full blood contained one minimal lethal dose. In other words, the guinea pig received 0.1 cubic centimeter of the toxin subcutaneously and at least 0.0005 cubic centimeter was circulating in every 0.5 cubic centimeter of its full blood. If we calculate the amount of total blood of the guinea pig of 300 grams body weight as approximately 20 cubic centimeters, fifty minimal lethal doses were circulating in its blood within twenty-four hours, which is one-fourth of the total amount of toxin injected.

From unpublished experiments performed some years ago by A. P. Hitchens and Otto Schöbl, we know that tetanus antitoxin injected into horses subcutaneously will appear in its maximum amount toward the end of the third or the fourth day after injection, and will not exceed 40 per cent of the calculated concentration in the blood. It is, therefore, at once evident that the resorption of the tetanus toxin is much quicker than the resorption of the tetanus antitoxin, particularly the concentrated globulin, which was used in our unpublished experiments. Theoretically, two hundred minimal lethal doses should be neutralized by two units of tetanus antitoxin, whereas of the two hundred minimal lethal doses subcutaneously injected fifty minimal lethal doses appeared in the circulating blood within twenty-four hours. The result of subcutaneous injection of two units would be that only 0.8 of a unit would circulate in the blood of the animal, and that not until the third day after the injection of the antitoxin. Therefore, in a case such as ours, in order to neutralize the tetanus toxin that circulates in the blood twenty-four hours after inoculation

with tetanus toxin, at least ten antitoxic units would have to be injected two days previous to the injection of two hundred minimal lethal doses.

In view of the fact that some sera are being tested with a view of learning their curative value and, furthermore, in view of the fact that tetanus toxin and antitoxin allow approximate mathematical calculation, this information is of practical value to anyone who wishes to test sera for their curative value.

ANILIDES AND TOLUIDES OF CHAULMOOGRIC ACID

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INTRODUCTION

The pioneer work on the constituents of chaulmoogra oil and the chemistry of the fatty acids present in it was carried out by Power and his associates.¹ They prepared the methyl and ethyl esters and a number of salts of chaulmoogric acid, one of the active constituents of chaulmoogra oil. Perkins² prepared the propyl, butyl, and amyl esters of the mixed chaulmoogra acids. Herrera-Batteke and West³ prepared the capryl, allyl, phenyl, ortho, meta, and para cresol esters of chaulmoogric acid. Aside from these and a few mercury derivatives, very little has been done on the line of derivatives of chaulmoogric acid.

Since chaulmoogric acid is a compound of high molecular weight it is much easier to prepare its amide than either the acid chloride or anhydride. Power and Gornall⁴ prepared chaulmoogramide from the acid chloride of chaulmoogric acid, following the general method of Aschan.⁵

The anilide of chaulmoogric acid may be prepared readily by fusing one mole of chaulmoogramide with a little over one mole of aniline at 195 to 200° C. Instead of aniline the toluidines may be used with equal success. By using substituted anilines the corresponding substituted anilides are obtained.

EXPERIMENTAL DATA

The chaulmoogra oil used in this investigation was obtained from the variety of chaulmoogra seeds known as *Hydnocarpus alcala* C. de Candolle, a tree indigenous to Albay, Philippine Islands, and known locally as *dudu dudu*. A sample of the oil

¹ Power, F. B., and F. H. Gornall, Journ. Chem. Soc. Trans. 85¹ (1904) 838 and 851; Barrowcliff, M., and F. B. Power, *ibid* 91¹ (1907) 557.

² Philip. Journ. Sci. 24 (1924) 621.

³ Philip. Journ. Sci. 31 (1926) 161-168.

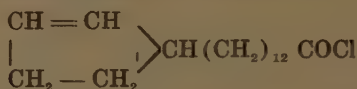
⁴ Journ. Chem. Soc. Trans. 85¹ (1904) 851.

⁵ Ber. 31 (1898) 2344.

was kindly presented to this laboratory by Dr. G. A. Perkins, chief chemist of Cullion Leper Colony.

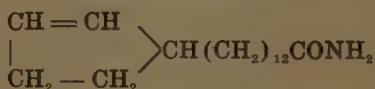
Chaulmoogric acid, $C_{17}H_{31}COOH$, was prepared according to the directions of Herrera-Batteke and West.⁶ The acid was crystallized from 95 per cent ethyl alcohol and then from petroleum ether (boiling point below $75^{\circ}C.$) to precipitate the resins, and finally from 95 per cent ethyl alcohol. Colorless glistening plates, which melted at $68^{\circ}C.$, were obtained.

ACID CHLORIDE OF CHAULMOOGRIC ACID



Sixty grams of chaulmoogric acid were placed in a round-bottomed flask provided with a reflux condenser and warmed until the acid melted; 16 grams of phosphorous trichloride were then added slowly from a dropping funnel and mixed thoroughly with the acid. The mixture was warmed with a small flame. The reaction was finished in fifteen minutes. Phosphorous acid separated at the bottom of the flask and by cooling with ice water became viscous, while the acid chloride remained as a light supernatant liquid. The acid chloride was poured into a dropping funnel and used in the preparation of chaulmoogramide.

CHAULMOOGRAMIDE



In preparing chaulmoogramide concentrated ammonium hydroxide was poured into a 1-liter beaker immersed in a freezing mixture. The acid chloride of chaulmoogric acid was allowed to drop slowly from a dropping funnel into the cold ammonia, while the mixture was stirred continuously. The amide separated out as a white crystalline precipitate. The amide was filtered and washed with water until the washings were no longer alkaline. It was then dried on sheets of filter paper, after which it was crystallized from 95 per cent ethyl alcohol. Colorless crystals, which melted at $104^{\circ}C.$, were obtained. The yield was 80 per cent. The amide was soluble in ethyl alcohol but insoluble in petroleum ether.

⁶ Philip. Journ. Sci. 31 (1926) 161-168.

The nitrogen content of the amide and of the other nitrogen compounds recorded in this paper was determined by Meulen's catalytic method.⁷

Analysis of chaulmoogramide.

Calculated for $C_{18}H_{33}ON$	Nitrogen. Per cent.
Found:	5.02
I	5.08
II	5.01

CHAULMOOGRANILIDE



In preparing chaulmoogranilide 40 grams of chaulmoogramide were placed in a round-bottomed flask provided with an air condenser and heated in an oil (crisco) bath to a temperature of 130° C.; 16 grams of freshly distilled aniline were added and mixed with the amide. The mixture was then heated at a temperature of 195 to 200° C. for five hours. The reaction started at 195° C., at which temperature ammonia gas was given off. The melt was then poured into a beaker of ice water and the anilide separated out as a crystalline mass. The anilide was filtered, washed thoroughly with water, and crystallized several times from alcohol (95 per cent) containing a small quantity of animal charcoal. It was obtained as colorless plates which melted at 89° C. The yield was 90.4 per cent. The anilide dissolved readily in ether, chloroform, and carbon tetrachloride, but was practically insoluble in petroleum ether and water. When hydrolyzed with sodium hydroxide and then heated with a few drops of chloroform and a slight excess of alcoholic potash, the odor of phenyl isocyanide was detected.⁸

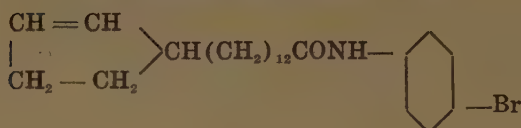
Analysis of chaulmoogranilide.

Calculated for $C_{26}H_{47}NO$	Nitrogen. Per cent.
Found:	3.94
I	3.86
II	3.70

⁷ Recueil des Travaux Chimiques des Pays-Bas 43 (1924) 643.

⁸ Hofmann's test for primary amines.

CHAULMOOGRA PARA BROM-ANILIDE

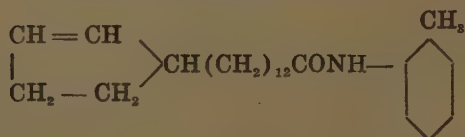


Nineteen and a half grams of chaulmoogramide were placed in a round-bottomed flask provided with an air condenser and heated in an oil (crisco) bath until the amide melted; 12 grams of para brom-aniline were then added and the mixture heated at 225° C. for half an hour. Ammonia gas was evolved. The reaction product was poured into a beaker of ice water and the precipitated brom-anilide was filtered off and washed with water. By repeated crystallization from 95 per cent ethyl alcohol, containing a small quantity of norit, colorless plates which melted sharply at 100° C. were obtained. They were soluble in ether, chloroform, and acetone, but practically insoluble in petroleum ether. The yield was 52.7 per cent. Qualitative tests showed the presence of carbon, hydrogen, nitrogen, and bromine.

Analysis of chaulmoogra para brom-anilide.

	Nitrogen. Per cent.
Calculated for $\text{C}_{29}\text{H}_{38}\text{ONBr}$	3.23
Found:	
I	3.38
II	3.29

ORTHO CHAULMOOGRATOLUIDE



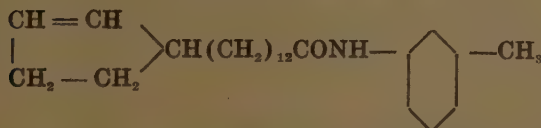
In the preparation of ortho chaulmoogratoluide 30 grams of chaulmoogramide were placed in a round-bottomed flask provided with an air condenser and heated on an oil (crisco) bath to 130° C. to melt the amide; 14 grams of freshly distilled ortho toluidine were then added and the mixture heated at a temperature of 230 to 235° C. for one and a half hours. Ammonia gas was evolved. The reaction product was then poured with constant stirring into a beaker of ice water. The toluidine precipitated out as a crystalline mass. This was filtered and washed with distilled water until free from ammonia. It was then crystallized repeatedly from 95 per cent ethyl alcohol,

containing a small quantity of norit, until the melting point was constant. The toluide was obtained as colorless plates, which melted at 95° C. The yield was 85.7 per cent. The toluide was soluble in the usual organic solvents, but insoluble in petroleum ether.

Analysis of ortho chaulmoogratoluide.

	Nitrogen. Per cent.
Calculated for $C_{27}H_{40}ON$	3.79
Found:	
I	3.67
II	3.77

META CHAULMOOGRATOLUIDE

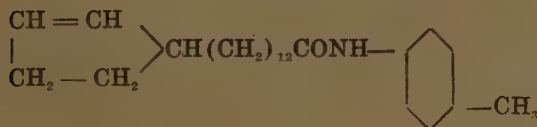


Forty grams of chaulmoogramide were placed in a round-bottomed flask provided with an air condenser and heated on a wire gauze until the amide melted; 18 grams of freshly distilled meta toluidine were then added and the mixture boiled gently for two hours. The apparatus was disconnected and the reaction product poured with constant stirring into a beaker of ice water. A colorless precipitate of the toluide was obtained. This was filtered, washed with distilled water, and dried between sheets of filter paper. It was then crystallized from 95 per cent ethyl alcohol containing a small quantity of norit. Colorless plates melting at 88° C. were obtained. The toluide was soluble in alcohol, ether, and chloroform, but practically insoluble in petroleum ether and water. The yield was 58.6 per cent.

Analysis of meta chaulmoogratoluide.

	Nitrogen. Per cent.
Calculated for $C_{27}H_{40}ON$	3.79
Found:	
I	3.89
II	3.76

PARA CHAULMOOGRATOLUIDE



Forty grams of chaulmoogramide and 18 grams of para toluidine were mixed in a round-bottomed flask provided with an air condenser and boiled gently on a wire gauze for two hours. The reaction product was then poured with constant stirring into a beaker of ice water. The precipitated toluidine was filtered, washed, and crystallized from 95 per cent ethyl alcohol containing a small quantity of norit. Colorless plates, melting at 100° C., were obtained. The yield was 51 per cent. The toluidine was soluble in alcohol, acetone, and chloroform, but practically insoluble in petroleum ether.

Analysis of para chaulmoogratoluidine.

Calculated for $C_{22}H_{25}ON$	Nitrogen. Per cent.
Found:	3.79
I	3.68
II	3.63

SUMMARY

1. Anilides and toluides of chaulmoogric acid can be prepared in fairly good yield from chaulmoogramide by treating it with the calculated quantity of amine base.

2. The following new derivatives of chaulmoogric acid have been prepared: Chaulmoogranilide, chaulmoogra para bromanilide, ortho chaulmoogratoluidine, meta chaulmoogratoluidine, and para chaulmoogratoluidine.

ACKNOWLEDGMENTS

I wish to express my thanks to Dr. A. P. West for his kind and helpful interest in this work and to Miss Paz Soriano for assistance in making the analyses.

AN ODORIFEROUS OIL AND TWO NEW LINOLIC TETRABROMIDES FROM PHILIPPINE LUMBANG OIL

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Philippine lumbang (candlenut) oil is a drying oil which is used in making paints, varnishes, and similar products.¹ A preliminary investigation of lumbang oil was made by West and Montes,² who showed that it consists principally of a mixture of unsaturated glycerides (linolenic, linolic, and oleic). The mixed unsaturated acids, obtained from the glycerides, were separated from each other by converting them into their bromo-derivatives³ which may be separated by solvents. The following four bromo-derivatives were thus prepared from lumbang oil:

Linolenic hexabromide, melting point, 178.5° C.

Linolic tetrabromide, crystals, melting point, 112 to 113° C.

Linolic tetrabromide (oil).

Oleic dibromide (oil).

In making this investigation the idea was to separate out the principal constituents. Only a very small quantity of oil was used in making the analysis, and no attempt was made to isolate minor constituents which may represent only a small proportion of the oil.

Some experiments carried out by us recently indicated that other constituents might be obtained from lumbang oil, in addition to those noted by West and Montes in their preliminary investigation. The results of this work showed that an odoriferous oil and also two crystallized tetrabromides (melting point

¹ West, A. P., and F. L. Smith, *Bull. P. I. Bur. Forestry* 24 (1923).

² *Philip. Journ. Sci.* 18 (1921) 619.

³ Lewkowitsch, J., *Chemical Technology and Analysis of Oils, Fats, and Waxes* 1 (1921) 585.

below 112° C.) could be obtained from lumbang oil. These low-melting tetrabromides are different from the linolic tetrabromide (melting point, 112 to 113° C.) prepared by West and Montes.

Takahashi⁴ prepared linolic tetrabromide (melting point, 114° C.) from soy-bean oil. When this tetrabromide was reduced to linolic acid and again brominated, he obtained three isomeric tetrabromides, which he designated as follows:

Compound.	Melting point. °C.
Alpha tetrabrom stearic acid (Alpha linolic tetrabromide)	113.5-114
Beta tetrabrom stearic acid (Beta linolic tetrabromide)	59-60
Gamma tetrabrom stearic acid (Gamma linolic tetrabromide)	(liquid)

In order to distinguish the isomeric tetrabromides obtained from lumbang oil, we used the nomenclature adopted by Takahashi for the corresponding compounds obtained from soy-bean oil.

EXPERIMENTAL PROCEDURE

Odoriferous oil.—Preliminary experiments showed that, when lumbang is steam distilled, apparently none of the oil distills over. If the aqueous distillate is extracted with ether, dehydrated, filtered, and the ether removed by distilling, a few cubic centimeters of a colorless oil are obtained. This oil has an intense lumbang odor and appears to be the substance that gives the characteristic odor to lumbang oil. The oil is very soluble in ether and dissolves when treated with a sufficient quantity of water.

In order to prepare a larger quantity of this odoriferous oil various methods were tried. The best method seemed to be to precipitate the acids, which occur as glycerides in the oil, in the form of zinc soaps. The zinc soaps were then separated by filtering and the filtrate extracted with ether, to obtain the odoriferous oil. Several batches of oil were treated in this manner.

This method was carried out according to the following procedure: The lumbang oil (600 grams) was saponified with aldehyde-free alcoholic potassium hydroxide in the usual manner. The alkali solution used for saponification was prepared

⁴ Journ. Tokyo Chem. Soc. 40 (1919) 233.

by treating 180 grams of potassium hydroxide with 80 cubic centimeters of water, after which 800 cubic centimeters of aldehyde-free alcohol were added. The mixture of oil and alkali solution was heated on a water bath (reflux) for about four hours to complete the saponification. The mixture was then cooled to room temperature and treated gradually with glacial acetic acid until the solution was about neutral to phenolphthalein which was used on a spot plate as an outside indicator. The mixed potassium soaps were treated with an alcoholic solution of zinc chloride containing a slight excess of the calculated amount of zinc chloride. Since the best grades of zinc chloride are sometimes contaminated with zinc oxide, it is advisable to ascertain how much zinc oxide is present in the zinc chloride. The estimated amount of zinc chloride is then treated with alcohol and the zinc oxide impurity removed by filtering. After adding the zinc chloride solution the mixture was heated on a water bath (reflux) for about two hours and then divided into four equal portions, each of which was poured into a separate large beaker. The mixtures were cooled in ice water and diluted with a sufficient amount of alcohol in order that the crystallized zinc salts might be filtered easily. The zinc salts were removed by filtering and the filtrate was concentrated, to obtain a second crop.

The filtrate from the second crop of zinc salts was poured into water, stirred thoroughly, and filtered. The aqueous filtrate was then extracted with ether. The precipitated zinc salts were washed thoroughly with water. The wash waters were extracted with ether and this ether extract was added to the first extract. The combined ether extracts were dehydrated with sodium sulphate, filtered, and the ether was removed by distilling. The crude odoriferous oil, which had a brown color, was distilled on an oil bath. It passed over as a colorless oil (boiling point, 80 to 95° C.), leaving a brown residue in the flask. When the colorless odoriferous oil was redistilled the boiling point was found to be 80 to 84° C. The oil had an intense lumbang odor, and the yield was about 2 per cent.

When the oil was treated with a glacial acetic acid solution of benzidine a decided orange color was obtained, indicating the presence of aldehydes. The oil decolorized a solution of pale, yellow, dilute bromine water and also a dilute solution of alkaline potassium permanganate, indicating that the oil contained unsaturated compounds.

The refractive index was determined at 20° C., and found to be 1.3638.

The oil is soluble in ethyl alcohol, methyl alcohol, ether, acetone, benzyl alcohol, glycerol, amyl alcohol, ethyl acetate, glacial acetic acid, and propyl and isopropyl alcohols. When treated with water it forms a turbid solution, but further addition of water gives a clear solution. It is insoluble in chloroform, benzene, xylene, nitrobenzene, toluene, carbon tetrachloride, and gasoline.

Mixed acids.—The zinc salts, prepared as described above, were treated with hot ethyl alcohol (95 per cent). A small quantity of a heavy oil separated out at the bottom of the alcoholic solution. The oil, when separated from the supernatant alcoholic solution and cooled, changed to a white solid. Investigation showed that this material consisted largely of oxidized zinc salts.

The alcoholic solution of unoxidized zinc salts was allowed to crystallize. The zinc salts were dried first on layers of filter paper and later in a vacuum desiccator. The weight of the zinc salts was ascertained, and they were then placed in a round flask which was fitted with a stopper containing three holes. In one hole was placed a tube which served to introduce carbon dioxide into the flask. A dropping funnel was passed through the middle hole, and through the third hole was passed a tube connected to a Bunsen safety valve. About 150 cubic centimeters of water were then poured into the flask through the dropping funnel. Carbon dioxide gas was generated by treating marble with dilute hydrochloric acid (1 : 3). The gas was purified by passing through two wash bottles containing sodium carbonate solution (which removed the hydrochloric acid vapors), and dried by passing through a wash bottle containing concentrated sulphuric acid and two bottles containing calcium chloride. The purified carbon dioxide was passed into the flask containing the mixed lumbang zinc soaps. The flask was then placed in a boiling-water bath and after the soaps had melted somewhat a dilute sulphuric acid solution (1 : 3) was introduced at intervals into the flask through the dropping funnel. After each addition of sulphuric acid the flask was shaken thoroughly. The zinc salts were thus gradually converted into the free mixed acids in an atmosphere of carbon dioxide. The mixture was cooled to room temperature. The mixed acids were then extracted with ether, and the ether extract was washed several times with distilled water and the

extract was then dehydrated with sodium sulphate and filtered. The weight of mixed acids contained in the ether solution was calculated from the known weight of zinc salts used.

Linolenic hexabromide.—The ethereal solution of mixed acids was diluted with a sufficient amount of ether to make the concentration one part of mixed acids to eight parts of ether. The diluted ethereal solution of mixed acids was treated with a slight excess of the calculated amount of bromine, according to the procedure used by Imperial and West.⁵ The ether solution of mixed acids was stirred mechanically by means of a hot-air motor and brominated at -10° C. The insoluble linolenic hexabromide was removed by filtering. After crystallizing from ethyl acetate and benzene, the melting point of the hexabromide was 179.5 to 180.5° C.

Analysis of the linolenic hexabromide gave the following results:

	Bromine.
Calculated for $C_{18}H_{26}O_2Br_6$	Per cent.
Found	63.32
	63.02

Alpha linolic tetrabromide.—The ethereal filtrate from the hexabromide was treated with sodium thiosulphate solution, to remove the bromine, and was then separated, dehydrated with anhydrous sodium sulphate, and filtered. The solution was then distilled to eliminate the ether. In order to facilitate the removal of the last portion of ether the mixture was distilled on a water bath (60° C.) and a current of carbon dioxide passed into the distilling flask. The residue was treated with cold petroleum ether which precipitated a mixture of linolic tetrabromides. The mixed tetrabromides were washed with petroleum ether, which removed principally the liquid oleic dibromide and also some liquid tetrabromide. The crude crystalline product was then dried on layers of filter paper. The tetrabromide crystals were somewhat yellow and they were also rather sticky and gummy, due to the fact that they were saturated with oils which were somewhat insoluble in petroleum ether. After crystallizing from ethyl alcohol the melting point was 110 to 113° C. The alcoholic filtrate from the crystallized tetrabromide was distilled until about a quarter of the alcohol

⁵ Philip. Journ. Sci. 31 (1926) 441-449.

was removed. When the remainder of the solution was cooled a second crop of tetrabromide (melting point, 110 to 113° C.) was obtained. The filtrate from the second crop of crude tetrabromide was set aside for further investigation.

The combined yields of tetrabromide were washed again with petroleum ether and crystallized once from gasoline and several times from ethyl alcohol. After this further purification the melting point was 112.3 to 114.3° C.

Analysis of alpha linolic tetrabromide showed the following results:

	Bromine.
Calculated for $C_{18}H_{32}O_2Br_4$	Per cent. 53.33
Found	53.73

This substance which, in accordance with the nomenclature adopted by Takahashi, we called alpha linolic tetrabromide, was the same as the tetrabromide prepared by West and Montes.⁶

Gamma linolic tetrabromide.—When the alcoholic filtrate from the second crop of alpha tetrabromide was concentrated, by distilling off about a third of the remaining alcohol, a red oily layer separated out at the bottom of the alcoholic solution. The red oil was then separated from the supernatant alcoholic solution of the alpha compound. When the red oil was shaken with a small quantity of cold ethyl alcohol a considerable portion of it gradually crystallized and was converted into silky crystals. The crystallization can be hastened somewhat by cooling the mixture in ice water.

That portion of the red oil which did not crystallize was analyzed and gave the following results:

Analysis of gamma linolic tetrabromide (red oil filtrate from silky crystals).

	Bromine.
Calculated for $C_{18}H_{32}O_2Br_4$	Per cent. 53.33
Found	53.37

According to the analysis, this red oil would thus appear to be a liquid tetrabromide and may, perhaps, be identical with the liquid tetrabromide (gamma linolic tetrabromide) described by Takahashi.⁷ Our liquid tetrabromide we called gamma linolic tetrabromide.

⁶ Philip. Journ. Sci. 18 (1921) 619.

⁷ Journ. Tokyo Chem. Soc. 40 (1919) 233.

Silky crystals (mixture of two substances).—The silky crystals obtained from a portion of the red oil were washed several times with cold ethyl alcohol. They melted at 59.6 to 60.6° C.

Analysis of the silky crystals gave the following results:

	Bromine.
Calculated for $C_{12}H_{22}O_2Br_4$	Per cent.
Found	53.33
	53.32

These silky crystals appeared to be a low-melting tetrabromide. The crystals were soluble in cold ether, chloroform, benzene, xylene, acetone, benzyl alcohol, nitrobenzene, toluene, carbon tetrachloride, gasoline, ethyl acetate, glacial acetic acid, and petroleum ether.

When the silky crystals were treated with warm methyl alcohol, a colorless oil separated out at the bottom of the alcoholic solution. The colorless oil was separated from the supernatant alcoholic solution and, when cooled, it changed to an amorphous white solid. The alcoholic solution when cooled with ice water gave a considerable quantity of white crystals. This behavior of the silky crystals indicated that they were a mixture of two substances, one of which was more soluble in warm methyl alcohol than the other. The silky crystals consisted of approximately 75 per cent of the very soluble substance which crystallized from the methyl alcohol solution and 25 per cent of the amorphous white solid that was not so readily soluble in methyl alcohol. Weighed quantities of each of these substances were treated with a sufficient amount of cold methyl alcohol to dissolve each of them. When calculated on the basis of 100 cubic centimeters of solvent, the results showed that 100 cubic centimeters of cold methyl alcohol, at room temperature (27° C.) dissolved 0.7463 gram of the crystallized substance and only 0.2164 gram of the amorphous substance.

Beta linolic tetrabromide.—The crystallized substance which was readily soluble in warm methyl alcohol was crystallized from the methyl alcohol solution. The melting point was 59 to 60° C. When observed under the microscope the crystals appeared as bundles of rods. We called this substance the beta linolic tetrabromide.

Analysis of beta linolic tetrabromide.

	Bromine.
Calculated for $C_{18}H_{32}O_2Br_4$	Per cent.
Found	53.33
	53.44

This beta linolic tetrabromide may be identical with the beta linolic tetrabromide (melting point 59 to 60° C.) obtained by Takahashi ⁸ from soy-bean oil.

Delta linolic tetrabromide.—That portion of the silky crystals which was not so readily soluble in methyl alcohol (the colorless oil that changed to a white solid) gave a melting point of 57 to 58° C. When crystallized from ethyl alcohol and viewed under the microscope the crystals appeared as bundles of needles. We called this substance delta linolic tetrabromide.

Analysis of delta linolic tetrabromide.

Calculated for $C_{18}H_{32}O_2Br_4$
Found

Bromine.
Per cent.
53.33
53.28

This delta linolic tetrabromide may be the same as the tetrabromide crystals (melting point, 57 to 58° C.) obtained by Matthes and Boltze ⁹ from the oil of wallflower seeds.

Our experiments would seem to indicate that we had prepared from lumbang oil not only linolenic hexabromide, oleic dibromide, and an odoriferous oil, but also four different linolic tetrabromides. Data on the tetrabromides are summarized in Table 1.

TABLE 1.—*Linolic tetrabromides from lumbang oil.*

Name.	Melting point.	Bromine content, ^a	Solubility in 100 cubic centimeters of cold methyl alcohol at 27° C.	Crystal form.
		°C.	Per cent.	
Alpha.....	112.3–114.3	53.78	Not determined.	Not determined.
Beta.....	59 – 60	53.44	0.7463	Rods in bundles.
Delta.....	57 – 58	53.28	0.2164	Needles in bundles.
Gamma.....	Liquid.	53.37	Not determined.	Liquid.

^a Bromine content calculated for $C_{18}H_{32}O_2Br_4$ (linolic tetrabromide) is 53.33 per cent. The temperatures recorded were corrected.

The tetrabromide silky crystals which were a mixture consisting of about 75 per cent of the beta compound and 25 per cent of the delta compound gave a melting point of 59.6 to 60.6° C. This is somewhat higher than the melting point of the delta compound, which is only 57 to 58° C. Beta and delta linolic tetrabromides were mixed in the proportion of 75 per cent of the beta and 25 per cent of the delta. The melting point of this mixture was found to be 59 to 60° C., which is identical with the melting point of the beta compound and approximately

⁸ Journ. Tokyo Chem. Soc. 40 (1919) 233.

⁹ Archiv der Pharmazie 250 (1912) 225.

the same as the melting point of the silky crystals prepared from lumbang oil.

Takahashi¹⁰ prepared alpha linolic tetrabromide (melting point, 114° C.) and reduced it to the free linolic acid which was again brominated. He thus obtained three isomeric tetrabromides. From his work it would appear that the elimination and, perhaps, the readdition of bromine gave molecular rearrangements which produced isomeric tetrabromides.

In our experiments with lumbang oil, the four isomeric tetrabromides were prepared by brominating directly the mixed acids which occur as glycerides in lumbang oil. There was no reduction of tetrabromides and rebromination of linolic acids.

Linolic acids.—From our results it would appear that lumbang oil may contain, perhaps, four different linolic glycerides. When these mixed glycerides are converted into the free linolic acids and brominated, each acid yields a particular tetrabromide which is different from the other tetrabromides.

If the formula "

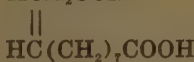


is accepted as the correct formula of linolic acid, then four space isomers of such a substance are possible. The formulas of these four isomeric linolic acids can be represented as follows:



trans-trans

I



cis-cis

II



cis-trans

III



trans-cis

IV

¹⁰ Journ. Tokyo Chem. Soc. 40 (1919) 233.

¹¹ Beilsteins Handbuch der Organischen Chemie, Vierte Auflage, 2 (1920) 496.

From the results of their experiments on cottonseed oil, Nicolet and Cox¹² were inclined to think that the *trans-trans* and *trans-cis* linolic acids were the only linolic acids occurring naturally as glycerides in cottonseed oil.

DISCUSSION OF RESULTS

Our results seem to indicate that, perhaps, lumbang oil contains all four linolic acids which are present as glycerides in the oil. If this be so, then, when the glycerides of lumbang oil are converted into mixed acids and these acids brominated, we should obtain linolenic hexabromide, four linolic tetrabromides (α , β , δ , and γ), and oleic dibromide. It appears that we have prepared all of these substances in this investigation. In making these compounds the insoluble hexabromide is first removed by filtering. After eliminating the ether solvent, the tetrabromides are precipitated with petroleum ether and removed by filtering. The petroleum ether filtrate from the crystallized tetrabromides consists principally of liquid linolic tetrabromide (γ) and oleic dibromide, as shown by West and Montes.¹³ We found that, when this filtrate was allowed to stand a few weeks, some crude crystals separated out. Investigation showed that these mixed crystals consisted largely of β and δ tetrabromides and, also, a small quantity of the α compound.

As previously stated, the crude α linolic tetrabromide, when first precipitated and washed with petroleum ether, appeared to be saturated with heavy oils. When this tetrabromide was crystallized from ethyl alcohol, and the filtrate from the second crop of crystals concentrated, a red oil separated out. When this red oil was removed and treated with ethyl alcohol, a considerable portion of it was gradually converted into silky crystals, which consisted of a mixture of two crystallized tetrabromides (β and δ). These tetrabromides can be separated by treating them with methyl alcohol, which dissolves the β compound more readily than the δ compound.

We thought that, possibly, the silky crystals were formed from the α tetrabromide by a molecular rearrangement and the red oil which separated out was a transition stage in the transformation of the α into the β and δ compounds.

¹² Journ. Am. Chem. Soc. 44 (1922) 144.

¹³ Philip. Journ. Sci. 18 (1921) 619.

In order to test this point, we dissolved some of our purest alpha tetrabromide in ethyl alcohol. The solution was allowed to crystallize. The filtrate from the second crop was concentrated, but no red oil separated out. The red oil is obtained only by concentrating the alcoholic filtrate from the precipitated crude alpha compound prepared directly from lumbang oil.

Our results would seem to indicate that, when the mixed tetrabromides are first precipitated with petroleum ether, the beta and delta compounds are obtained as heavy oils which adhere tenaciously to the crystallized alpha compound. There is also a small amount of the oily gamma compound in the mixture as well as some oleic dibromide. The mixed bromides are washed with petroleum ether to eliminate the oily oleic dibromide and also some liquid tetrabromide, and then they are crystallized from ethyl alcohol. The filtrate from the second crop of the crystallized alpha compound is a dilute alcoholic solution of the alpha, beta, delta, and gamma compounds. By concentrating this filtrate, the beta, delta, and gamma compounds separate out as oils, because these substances are insoluble in a concentrated alcoholic solution of the alpha compound. When these oils are separated from the supernatant alcoholic solution of the alpha compound, the beta and delta compounds are gradually changed to silky crystals. The addition of some alcohol at this stage facilitates the crystallization, because the alcohol tends to dissolve the small quantity of gamma compound which is present.

In order to test our idea as to how the beta and delta compounds were obtained, we dissolved small portions of the individual purified alpha, beta, delta, and gamma compounds in ethyl alcohol. When the alcoholic solution of these four compounds was concentrated and cooled the red oil separated out as usual. When the red oil was separated from the supernatant alcoholic solution of the alpha compound and treated with a small quantity of ethyl alcohol, a considerable portion of the oil was gradually converted into silky crystals.

The beta and delta compounds appear to be new substances, which have not been definitely described in the literature.

SUMMARY

Lumbang oil consists principally of a mixture of unsaturated glycerides (linolenic, linolic, and oleic). It also contains an odoriferous oil which has an intense lumbang odor and boils at 80 to 84° C.

The odoriferous oil is soluble in water and various organic solvents, and gives positive tests for aldehydes and unsaturated compounds.

In addition to linolenic hexabromide and oleic dibromide, four isomeric linolic tetrabromides may be obtained from lumbang oil. They are as follows:

Linolic tetrabromide.	Melting point. °C.
Alpha	112.3-114.3
Beta	59-60
Delta	57-58
Gamma	Liquid.

By concentrating the alcoholic filtrate from the alpha compound, a red oil is obtained. When isolated, a considerable portion of this red oil is gradually converted into silky crystals. These crystals consist of a mixture of beta and delta compounds, which may be separated by means of methyl alcohol. It appears that the individual beta and delta compounds have not been definitely described in the literature.

These four linolic tetrabromides may have been obtained, perhaps, from four different linolic acids which were present as glycerides in the lumbang oil.

ACKNOWLEDGMENT

We wish to express our thanks and appreciation to Mr. Frederick L. Smith, 2d, chief chemist, Quartermaster Corps, United States Army, Manila, who kindly made our bromine analyses for us.

TINGITIDÆ FROM THE FAR EAST (HEMIPTERA)

By CARL J. DRAKE

Of Iowa State College, Ames

Through the courtesy of C. F. Baker, dean of the College of Agriculture, University of the Philippines, and of other co-workers I have received for study collections of lace bugs from India, China, Japan, and the Philippine Islands. In classifying these collections two new genera and eight new species have been discovered, which are characterized herein.

MONANTHIA EVIDENS sp. nov.

Very similar to *M. formosa* Drake, but slender, much darker in color, the pronotum more evenly rounded on the sides, and the nervure between subcostal and discoidal areas at the apex of discoidal area (over tumid elevation) rounded and not angulate.

Antennæ testaceous, the basal half of the first segment slightly embrowned, the proportional lengths of the segments as in *M. formosa*. Rostrum reaching between the intermediate coxæ. Venter with two prominent, black, stout, blunt, obliquely projecting tubercles, one on each side of the penultimate segment. Head black; the posterior spines brownish, contiguous with the surface of the head, reaching to near the anterior margin of the eyes; median and anterior spines greatly reduced, represented by very short, blunt, black tubercles. Pronotum and paranota black, the cells a little lighter, the lateral carina, if present, represented by short, black, oblique nervures. Legs testaceous.

Elytra black, the areolæ mostly whitish but some of them partly embrowned; costal area narrower than in *M. formosa*, the transverse nervures on basal half black; subcostal area broad, triseriate; discoidal area with five areolæ at its widest part, the nervure between discoidal and costal areas curved, rounded at the tumid elevation.

Length, 2.7 millimeters; width, 1.02.

Holotype, female, Imugan, Nueva Vizcaya, Luzon (*Baker*), in Drake collection. Specimens of both *M. sauteri* Drake and *M. formosa* Drake are at hand from the Philippine Islands, here-

tofore recorded only from Formosa. *Monanthia evidens* is closely allied to *M. formosa*, but easily separated from it by the tubercles on the venter (projecting obliquely outward), the paranota, and the shape of the nervure between subcostal and discoidal areas at the apex of the latter.

ONCOPHYSA CONSTANTIS sp. nov.

Moderately elongate, stout, brown, with testaceous and fuscous markings. Head black; spines testaceous, blunt, stout, moderately long; posterior spines directed forward, touching the surface of the head, reaching to the anterior spines; median and anterior spines pointed slightly downward, the anterior pair with their tips almost touching. Rostral laminae very widely separated on the metasternum, the rostrum reaching to the mesometasternal suture.

Antennae long, brown; segment I stouter and nearly twice as long as the second; III long, slender; IV wanting. Pronotum tricarinate, strongly tumid through the disk; paranota formed as in *O. vesiculata* Stål, but more tumid, a little higher, not shiny, and the nervures more prominent.

Elytra testaceous brown, rather dull, with a number of fuscous cells in the sutural areas; costal area composed of one row of moderately large cells, with a dark band (two or three cells) at its widest part; discoidal area large, the outer margin slightly curved, areolae strong depressed; subcostal area biserrate, the areolae slightly smaller than those of discoidal area. Wings a little shorter than the elytra. Length, 4.24 millimeters; width, 1.16.

Holotype, female, Hongkong, China (*H. Koebele*), in Drake collection. This species may be separated from *O. vesiculosa* Stål by its shorter antennae, larger paranota, rather dull dorsal surface of the body, and costal area.

DIPLOCYSTA NIMIA sp. nov.

Elongate-ovate, broad, large, brown, with blackish markings. Head short, broad; median and anterior spines very short, blunt, posterior spines short, rather slender, touching the surface of the head, not reaching anterior spines. Bucculae closed in front. Antenniferous tubercles large, very broad, prominent.

Antennae long, rather slender, brown, the fourth segment (except a very small basal portion) black; segment I considerably larger and longer than the second; II very short, not very stout, less than half as long as I; III slender, slightly more than twice

the length of IV. Pronotum tricarinate, the entire dorsal surface above, except small median portion of collum and posterior triangular portion, concealed by the enormously developed and inflated paranota; median portion of triangular process raised, rooflike, the median carina placed on this rooflike ridge; lateral carinæ, except small apical portion, concealed by the paranota. Paranota enormously developed, strongly tumid, a little longer than broad, crests widely separated (deep V-shaped space between them); areolæ large, opaque, dull, a little lighter in color than the nervures; nervures brown, shiny.

Elytra very broad, rounded at the apex, broadest at the apex of pronotum (triangular process), testaceous brown, with fuscous or black markings, the apical portion fuscous; costal area broad, with outer margin somewhat curved, the areolæ moderately large, not arranged in regular rows, with a very broad, blackish fascia at widest portion; subcostal area broad, composed of five rows of small areolæ, with a long, prominent, costate nervure near the outer margin (separating one row of cells along the costal margin), composed of six rows of cells; discoidal area large, broad, strongly impressed, bounded by a prominent nervure, outer margin nearly straight, composed of nine or ten cells at its widest part. Length, 5.12 millimeters; width, 2.34.

Holotype, male, Cuernos Mountains, Negros (*Baker*), in Drake collection.

DIPLOCYSTA NUBILA sp. nov.

Form, size, and general appearance very much like *D. nimia* sp. nov., but distinguished from it by its dark, fuscous or blackish, narrowly separated paranota, the nonelevated median portion of the triangular process of the pronotum, and the narrower costal area of the elytra.

Antennæ long, segments I and II slightly shorter than in *D. nimia*. Paranota enormously developed, blackish fuscous, the great tumid structures deeply and narrowly separated, the crests not widely separated. Bucculæ closed in front.

Elytra a little narrower than in *D. nimia*, not so strongly marked with fuscous or black; costal area moderately broad, with three to four rows of areolæ, the areolæ more regularly arranged, with a narrower brown fascia near the middle; subcostal area with slightly larger areolæ, the row of cells between the costate nervure of subcostal area and costal area a little

larger; discoidal area distinctly impressed, composed of eight or nine cells at its widest part; sutural area much lighter than in *D. nimia*. Length, 4.7 millimeters; width, 2.04.

Holotype, female, Singapore (*Baker*), in Drake collection. This peculiar insect is a little smaller than and very distinct from *D. nimia* sp. nov.

Genus UHLERITES novum

Distinctly lacy, elongate, obovate. Head very short, with five spines, the posterior pair contiguous with surface of head. Bucculae closed in front.

Antennae long, slender; segment I stouter and longer than II; segment III very long and slender; segment IV longer than I, slightly enlarged toward the tip. Orifice prominent. Rostral channel uninterrupted. Pronotum strongly and transversely swollen through the disk; coarsely punctate, strongly narrowed anteriorly, unicarinate; humeri prominent. Hood moderately large, subangularly projecting over base of head. Paranota reticulate, moderately broad, angularly projecting in front.

Elytra reaching considerably beyond the apex of abdomen; costal and subcostal areas broad; discoidal area about reaching the middle of the elytra, the boundary between discoidal and subcostal areas distinctly raised.

Type of genus, *Uhlerites* (*Phyllontocheila*) *debile* Uhler, from Japan.

This genus is closely allied to *Gelchossa* Kirkaldy, from which it can be separated by the unicarinate pronotum, very short head, thicker nervures, and differently formed paranota and hood.

HORMISDAS VICARIUS sp. nov.

Coloration, form, and size very similar to *H. pictus* Distant. Hairs fine, slightly curled. Paranota moderately reflexed, biseriate, the outer margins nearly straight and not roundly emarginate on the sides in front, as in *H. pictus*, each armed with a long spine in front. Carinae slightly more raised than in *H. pictus*, the median carina more raised in front and the dorsal margin nearly straight. Spines on the head long, sharp, slenderer than in *H. pictus*. Costal area of elytra moderately broad, irregularly biseriate, a little narrower than in *H. pictus*, the nervure between subcostal and discoidal areas (near the middle), and the apex of discoidal area only slightly raised. Length, 2.9 millimeters; width, 1.2.

Four specimens. Holotype, male, Larat (*Muir*) in my collection. Paratypes, one, Larat (*Muir*); two, Los Baños, Philippines (*Baker*). In Baker and Drake collections.

The above description is made from comparison with a male cotype of *H. pictus* Distant.

Genus XENOTINGIS Drake (1923)

Xenotingis DRAKE, Ohio Journ. Sci. 23 (1923) 105.

Orthotype, *Xenotingis horni* Drake.

This genus is based upon a single species, *X. horni* Drake, from Formosa. A second species, described from a single specimen, slightly modifies the original description of the genus. The pronotum is uni- or tricarinate; the posterior triangular process is more or less produced. The areas of the elytra are not differentiated in the new species described below.

Key to species of *Xenotingis* Drake.

- Pronotum tricarinate, the triangular portion long and visible behind the enormously developed paranota; margins of paranota forming a large round opening above the pronotum..... *X. horni* Drake.
 Pronotum unicarinate, the triangular portion short and not quite reaching to the center of the paranota; margins of paranota forming a narrow opening above the pronotum..... *X. bakeri* sp. nov.

XENOTINGIS BAKERI sp. nov.

Narrow, elongate, testaceous, the pronotum enormously developed. Head short, testaceous, with five spines; posterior spines moderately long, blunt, directed forward, contiguous with the dorsal surface of the head; anterior spine not very long, appressed; anterolateral spines moderately long, semierect, their tips touching. Rostral channel widening posteriorly, the rostrum extending to the end of rostral channel. Bucculæ closed in front.

Antennæ long, slender, somewhat curved, the apical segment clothed with a few rather short hairs; segment I a little longer and stouter than II; III long, very slender, nearly four times the length of IV. Pronotum unicarinate, very much concealed by the extremely large paranota; posterior process very short, the apex bluntly rounded. Paranota enormously developed, somewhat globose, projected high above the pronotum and base of elytra, a little longer than high, the outer margins not widely separated above the pronotum; areolæ large, clouded with pale brown; apex of triangular process of pronotum not quite reaching to the middle of the paranota. Hood moderately large,

projecting a little over the base of the head, the median carina slightly raised anteriorly.

Elytra considerably longer than the abdomen, rounded at the apex, the outer margins (on each side) recurved; areas not differentiated, the areolæ rather large. Wings much shorter than the elytra but longer than the abdomen. Length, 3.15 millimeters; width, 0.8.

Described from a male specimen (holotype), Los Baños, Philippines (*Baker*), in my collection. This species is very distinct from *X. horni* Drake from Formosa.

XENOTINGIS HORNII Drake.

Xenotingis horni DRAKE, Ohio Journ. Sci. 23 (1923) 105, fig. 1.

This species was described from a female specimen from Kosempo, Formosa, collected by Mr. H. Sauter. It can be easily distinguished from *X. bakeri* by the characters of the paranota and pronotum.

Genus ALLOIOTHUCHA novum

Distinctly lacy, as in the genera *Stephanitis* Stål, *Gargaphia* Stål, *Leptobrysa* Stål, and *Corythucha* Stål. Rostral channel uninterrupted. Antennæ long, slender; segment III slenderest, only slightly longer than IV; segment IV very long. Bucculæ not contiguous in front. Intermediate and posterior coxæ widely separated. Head short, concealed from above by hood. The spines reduced. Pronotum and head entirely concealed by an enormous cyst, the reticulations rather large. Paranota narrow, areolate. Elytra widely reticulated, extending considerably beyond the apex of the abdomen, the tips rounded and widely separated; discoidal area not reaching the middle of the elytra.

The extremely large and strongly inflated cyst (covering the head, pronotum, paranota, and posterior process of pronotum) separates this genus from closely related genera.

Type of genus, *Alloiothucha philippinensis* sp. nov.

ALLOIOTHUCHA PHILIPPINENSIS sp. nov.

Testaceous, the enormous cyst embrowned, the cells of elytra hyaline. Head short, concealed by the hood. Rostrum reaching to the end of the rostral channel.

Antennæ slender, long, testaceous; segment I a little longer and slightly stouter than II; segment III only slightly longer than IV (III=38; IV=34); segment IV slightly swollen, clothed with a few short hairs. Pronotum entirely concealed by the

extremely large hood. Paranota narrow, biseriate, reflected almost vertically, the areolæ small. Body beneath brownish. Hood enormously developed, strongly inflated, ellipsoidal, a little longer than high; areolæ large, embrowned, nontransparent.

Elytra divaricating posteriorly, rather widely reticulated; costal area broad, with three rows of cells at its widest part, the areolæ of costal and discoidal area subequal in size; subcostal area uniseriate, the cells very small along the discoidal area; discoidal area short, widening posteriorly, not reaching the middle of the elytra, angulate behind, with four rows of cells at its widest part, the areolæ only slightly smaller than those of sutural area. Wings atrophied. Legs yellowish brown. Length, 3.1 millimeters; width, 2.

Holotype, male, Mount Maquilung, Luzon (*Baker*), in my collection.

ALLOIOTHUCHA NECOPINATA sp. nov.

Closely related to *A. philippinensis* sp. nov., but readily separated from it by the much shorter paranota and the wider costal area of the elytra. Length, 3 millimeters; width, 2.

Antennæ slightly stouter than in *A. philippinensis*; segment III distinctly longer than IV (III=44; IV=35). Paranota short, biseriate, not reaching to the posterior margin of humeri, the outer margin rounded. Hood ellipsoidal, a little longer than in *A. philippinensis*; areolæ smaller, embrowned, nontransparent. Rostrum long, reaching to the apex of rostral channel.

Elytra divaricating posteriorly, not as broadly rounded at the apex as in *A. philippinensis*; costal area broad, with four or five rows of cells at its widest part; discoidal area not reaching the middle of the elytra, rounded behind, with four rows of cells at its widest part; subcostal area uniseriate. Wings almost as long as the abdomen. Body beneath dark reddish brown.

Holotype, male, Puerto Princessa, Palawan (*Baker*), in my collection.

Key to species of Alloiouthucha g. nov.

Costal area of elytra triseriate at its widest part; the discoidal area angulate behind; paranota extending to the base of elytra.

A. philippinensis sp. nov.

Costal area with four or five rows of areolæ at its widest part, the apical margin of discoidal area rounded; paranota short, not reaching to the base of elytra..... *A. necopinata* sp. nov.

ZWEI NEUE ANOMALA-ARTEN DER PHILIPPINEN (COLEOPTERA, LAMELLICORNIA, RUTELIDEN)

Von F. OHAUS

Mainz, Germany

EINE TEXTFIGUR

ANOMALA FUSCAOAREA sp. nov.

Diese Art bildet einen Uebergang zwischen der *A. camariensis* Ohaus und *inconsueta* Ohaus. Gestreckt eiförmig, hinten leicht verbreitert, flach gewölbt. Kopf, Vorderrücken, Schildchen, die Unterseite und die Schienen nebst Tarsen sind dunkelbraun mit kupfrigem Bronzeschimmer, wenig glänzend, die Deckflügel, Afterdecke und die Schenkel hellbraun, die letzteren allein mit schwachen grünen und kupfrigen Lichtern. Oberseite kahl, die Spitze der Afterdecke und die Brust nebst Hüften mit spärlichen kurzen gelben Haaren. Kopfschild, Vorderrücken, und Schildchen sind dicht, vielfach zusammenfliessend und ziemlich grob punktirt, die basale Randfurche in der Mitte unterbrochen. Auf den Deckflügeln ist von der primitiven Sculptur nur noch die die Nahtrippe begrenzende primäre Punktreihe und von den primären Rippen kurze Reste der II. III. IV. und V. neben dem Spitzenbuckel erhalten; sonst sind die Deckflügel dicht bedeckt mit zusammenfliessenden grossen Ringpunkten, zwischen denen kurze Querrunzeln verlaufen, die ganze Oberfläche überstreut mit sehr feinen Pünktchen, zumal auf der Nahtrippe. Afterdecke mit grossen Ringpunkten und groben Querrunzeln, Unterseite (Bauch, Brust, und Hüften) mit einzelnen grossen Ringpunkten, die nur an den Seiten zusammenfliessen. Vorderschienen mit grossem löffelartigem Spitzenzahn und einem scharfen Seitenzahn; Mittel- und Hinterschienen mit je zwei schiefen Borstenreihen. Fühler hellbraun.

Länge, 21 Millimeter; Breite, 11.

Weib, von Samar (*C. F. Baker*).

EUCHLORA HORTENSIA sp. nov.

Der *E. latefemorata* Ohaus von Nord-Borneo zünächst verwandt. Gestreckt eiförmig, hinten leicht verbreitert. Ober-

seite und Afterdecke satt grasgrün, glänzend; Unterseite, Schienen, und Füsse erzgrün, alle Hüften und Schenkel braungelb mit lebhaftem rotem Kupferschiller; Fühler hellbraun. Kopf, Schildchen, und Vorderrücken fein einzeln punktirt, die Seiten des letzteren schmal gelb gesäumt und die basale Randfurche



FIG. 1. *Euchlora hortensia* sp. nov.; Forceps, dorsale und linke Seitenansicht.

in der Mitte unterbrochen. Die Deckflügel weitläufig fein punktirt mit regelmässigen primären Punktreihen, deren Punkte etwas grösser als die zerstreuten Pünktchen sind. Afterdecke kahl, sehr dicht und fein rissig punktirt, fast matt. Bauchringe lebhaft glänzend, mit der gewöhnlichen Querreihe von Borstenpunkten; Hüften und Brust kurz gelb behaart. Beine ohne Besonderheiten. Am Forceps, Fig. 1, sind die Parameren symmetrisch, frei, nicht so stark verschmälert als bei der *latefemorata*, die Ventralplatte des Mittelstückes in ihrer apicalen Hälfte schaufelförmig, ihr Vorder- rand schwach ausgeschnitten.

Länge, 21 Millimeter; Breite, 11.5.

Mann, Samar (*Baker*).

ILLUSTRATION

TESTFIGUR

FIG. 1. *Euchlora hortensia* sp. nov.; Forceps, dorsale und linke Seitenansicht.

IMPERFECT HERMAPHRODITISM IN FLOWERS OF HIBISCUS, REMOVED BY SURGICAL OPERATION ¹

By NEMESIO B. MENDIOLA

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the Philippines, Los Baños, Laguna*

FOUR PLATES

INTRODUCTION

In the course of my genetic studies on *Hibiscus*, there were discovered in this genus various degrees of sterility and fertility which seem to me of biologic interest. One of the sterile forms was found in the flowers of a certain variety of *H. rosa-sinensis*. The species is supposed to bear hermaphroditic buds and flowers; but in the case of the variety the stigmas, as a rule, owing to abnormal shortness of style, fail to reach even the terminal column when the flower is in bloom. We have here, then, a case of imperfect hermaphroditism. I do not know the origin of this variety; I am sure it is not one of our seedlings. Since Wilcox and Holt ² reported in 1913 that they obtained in Hawaii a hybrid with this peculiarity, and as many of our introduced varieties were imported from Hawaii, it is possible that the variety under discussion is identical with that of Wilcox and Holt. It will be of horticultural interest to ascertain if this is a case of a perfect hermaphrodite under foreign conditions becoming imperfect upon a change of environment. Until the identity of the variety under report is known, I am giving it the convenient variety name of Prisoner. It may be remarked that this variety is odorous. The case of protandrous sterility just described also occurs, though very rarely, in three other, quite distinct varieties in my collection; namely, the Woodrow Wilson, which is also odor-producing; the Two-Days, the flower of which, unlike those of other varieties, blooms for two days; and the Jamaica. A highly significant peculiarity of the Prisoner is that a plant of this variety, budded on a Native Red Single stock about 10 years

¹ Read before the Los Baños Biological Club, December 11, 1924.

² Ornamental Hibiscus in Hawaii, Bull. Hawaii Agr. Exp. Sta. 29 (1913).

old, produces mostly perfect hermaphrodites. Table 1 is a record of the condition of the flowers produced by the Prisoner from November 26, 1924, to February 9, 1925.

TABLE 1.—Showing the variability in distance between the uppermost stigma and opening of staminal column in flowers of the same age of *Hibiscus rosa-sinensis* var. Prisoner, collected November 26, 1924, to February 9, 1925.

Class range.	In flowers of a plant budded on another variety.	In flowers of plants growing on their own roots.
	Flowers.	Flowers.
Twelve to 17 millimeters above opening.....	3	0
Six to 11 above opening.....	54	4
Less than 6 above opening.....	36	14
At opening.....	1	11
Less than 6 millimeters below opening.....	2	52
Six to 11 below opening.....	3	15
Twelve to 17 below opening.....	0	1
Eighteen to 23 below opening.....	0	0
Twenty-four to 29 below opening.....	0	0
Thirty to 35 below opening.....	1	0
Thirty-six to 41 below opening.....	0	0
Forty-two to 47 below opening.....	0	1
Forty-eight to 53 below opening.....	0	1
No style ^a	1	1
Total.....	100	100

^a The length of the normal style of the variety is about 100 millimeters.

Table 1 shows the variability in distance between the uppermost stigma and the opening of the staminal column. It will be observed that, typically, the flower of Prisoner on a budded plant has its uppermost stigma about 6 to 11 millimeters above the opening. Ninety-three per cent of the flowers had stigmas above the opening; in other words, ninety-three per cent of the flowers were in position to function in pollination. On the other hand, plants growing on their own roots produced typically flowers with stigmas only within 6 millimeters of the opening of the staminal column. Seventy-two per cent of the flowers of such plants produced their stigmas below the opening of the column and were not in position to function in pollination.

It would appear that there is a tendency for Prisoner of both sexes growing on its own roots to be sterile; for, aside from the anatomical imperfection of its stigmas, its pollen is also physiologically incompatible with the stigmas of all the

fifteen varieties which I have pollinated with it and of five additional varieties pollinated by Mr. Unite, graduate assistant in plant breeding. Moreover, Prisoner is itself self-incompatible; that is, under conditions when it is female-fertile with other varieties, it is female-sterile with its own pollen.

It is to be noted again that the pollen from a budded plant of Prisoner was found fertile, or functional, with varieties on which pollen of the same variety but from plants growing on their own roots failed to function. For example: Variety Pink-10 was pollinated on November 6, 12, and 18, and December 5 and 9 with pollen from plants growing on their own roots, but none of the treated flowers produced fruit. The same variety, pollinated on December 4, 1924, with pollen from a budded Prisoner plant, produced a fruit which was harvested on January 9, 1925, and was found to contain three good seeds. Similarly, varieties Pink-14, Almost White, and Dwarf Pink were sterile to pollen from Prisoner plants growing on their roots, but fertile to pollen from a plant of the same variety budded on Native Red Single. The seeds obtained in these crosses were sown and found to be viable.

While the phenomenon of sterility may be profoundly concerned with theories of evolution of species, this work was undertaken not so much to secure evidence contributive to these theories as to attempt to induce fertility in a variety which is ordinarily self- and female-cross-sterile, so that I might study genetically its unique odoriferous quality.

SURGICAL OPERATION TO CORRECT IMPERFECT HERMAPHRODITY

How the operation was discovered.—One morning Mr. Unite, my assistant, brought to me three flowers of Prisoner, the stigmas of which were more or less exposed and which were more normal in appearance than those found in the staminal tube. The top of the column in these flowers had been removed by some agent. As I am somewhat familiar with the injuries which insects inflict on the flowers and flower buds of *Hibiscus*, I suspected at once that we had stumbled on a case of a surgical operation accidentally performed by insects which made possible normal development of otherwise anatomically functionless stigmas. As some time must have elapsed since the insect attacks for the stigmas to reach the mature stage, I further suspected that the injury to the terminal portion of the staminal tube must have taken place many hours before the flowers opened. I then decided to perform the following artificial surgical opera-

tion on the Prisoner flowers about twenty-four hours before they opened.

The surgical operation.—Essentially, this operation consists in splitting with a needle the terminal portion of the staminal tube which imprisons the stigmas and bending the split parts outward so as to expose the stigmas. The operation must be performed with care, so as not to injure the stigmas and the style while freeing the stigmas from the thin membrane covering them. It has been found, provided the work is done about twenty-four hours before the flower opens, that this operation is invariably effective in causing the stigmas to reach maturity and to attain a receptive condition by the time the flower blooms. Performing the operation immediately before pollination brought only partial success.

That essentially the same result is attained through insect attacks as by operation has already been pointed out. At least five species of insects have been observed feeding on flowers and flower buds of *H. rosa-sinensis* and any one of them should be capable of accomplishing the operation even in what is apparently an accidental way. These species³ are *Phaneroptera furcifera* Stål, *Phenacoccus hirsutus* Green, *Nisotra gemella* Erichson, *Nisotra* sp., and *Cosmophila erosa* Hübner.

FERTILITY OF STIGMAS EXPOSED THROUGH OPERATION

Through artificial operation.—It remains to show that stigmas released and exposed through this operation are functional. Stigmas of this sort were hand-pollinated on different days, both in my cultures at home by myself, and by Mr. Unite in our cultures in the plant-breeding garden. Using Pink-10 as male parent, several ripe fertile pods were produced on the Prisoner, thus proving the fertility of the stigmas exposed through surgical operation. Seeds of these pods have been sown and seedlings of the cross produced.

Through insect operation.—Two flowers, the stigmas of which were found barely out of the opening of the staminal column, were one day hand-pollinated with pollen of variety Pink-10. This pollination was also successful. However, I have no way of satisfying myself whether the stigmas of these flowers were out as a result of normal development or as the result of insect attack on the tip of the column. When the pollination was done the stigmas had emerged from the top of the column.

³ Identified by the department of entomology, College of Agriculture.

Whatever the case may be, there is no reason known at present why stigmas exposed accidentally should not be as potent as those exposed artificially. If this point be granted, we may assign a new, although apparently at present accidental, rôle to insects; namely, that of serving as agents in fruit production while performing their injurious work on the flowers. Perhaps in the case of the variety Prisoner (its stigmas being impotent anyway) the insects that eat off the staminal column are not to be classed as enemies; for, while they mutilate and devour the flowers, they nevertheless perform work really essential to the species, that of making reproduction possible. Up to date, Prisoner has not produced fruits that are the result of insect pollination. Other varieties, as our Unknown-3, Unknown-4, Unknown-7 (Pink \times Araña) F_1 -4, (Pink \times Araña) F_1 -5 and (Hawaiian Salmon \times Native Red) F_1 , have become fruitful through this agency. Several reasons may be assigned for the failure, thus far, of Prisoner to produce fruits in the open. One of these is that we have used almost daily practically all the flowers of our plants of this variety. Another reason is that the papilionid, *Ornithoptera nephereus* Gray, which according to my observation is the most efficient insect agent of pollination of *Hibiscus*, does not seem to care to visit *Hibiscus* flowers that are odoriferous, as is the case with those of the variety Prisoner. I have of course, found Prisoner flowers with pollen-covered stigmas on several occasions; but in these cases the wind might have served as a pollinating agent, not by carrying the pollen, as we ordinarily assume it to do, but by agitating the branches and causing the twigs and flowers to rub against each other, thus causing both autogamous and geitonogamous pollinations. In such a case, the pollen could have been that of Prisoner itself. Many *Hibiscus* varieties also would be capable of autopollination when the stigmas have just emerged. It has been pointed out that the pollen of Prisoner is impotent on its own stigmas. Besides autopollination caused by rubbing, I have also observed two other methods of autopollination.

WHEN TO PERFORM THE OPERATION

It has been found that flowers operated upon about twenty-four hours before blooming, but pollinated at the time of opening, set fruits almost invariably. Flowers operated upon and also pollinated twenty-four hours before blooming did not set

Pods. If operated upon and also pollinated at the time of blooming a small percentage of the flowers so treated produced fruits. It is quite probable that the proper length of time to be allowed between operation and pollination depends to a certain extent upon the relative stage of development of the style at the time the operation is performed. Those flowers which produced fruits invariably when operated upon about twenty-four hours before blooming and pollination, were the typical ones; namely, those in which the stigmas were only about 1 to 5 millimeters below the opening of the staminal tube.

SIGNIFICANCE OF THE PRESENT OBSERVATIONS

The results of the foregoing observations would seem to have an important bearing on organic evolution and on methods of plant breeding. From the latter standpoint, the importance of the results of operation and the setting of seeds of the flowers operated upon is obvious, and further discussion of this point is deemed unnecessary. Suffice it to say that a variety that is otherwise incapable of producing seeds has been forced to fruit. It is the theoretical bearing of the results that I desire to discuss more fully. It is generally believed that plants bearing hermaphrodite flowers, of which *Hibiscus* plants are examples, will become after a time dioecious individuals. Just which changes first, the morphology or the physiology of the sexual organs, is not well known. The case of flowers with hidden stigmas, reported in this paper, would apparently provide a point in support of the theory of morphological priority.

The present observations also have a bearing upon the effect of stock on scions in general, and on the sex of the latter in particular. That the stock affects its scion is well known in horticulture. The effect has been varied. Published results on this point, however, do not seem to include the effect of the stock on the sex of the scion. Cases of known degrees of barrenness and productivity induced by budding or grafting are common, but these phenomena are not necessarily dependent on the nature of sex organs. In the present work it has been pointed out that a variety of *H. rosa-sinensis*, which produces anatomically imperfect hermaphrodites and impotent pollen when grown on its own roots, produces perfect hermaphrodites and potent pollen when budded on another variety. This fact would support the theory that perfect or imperfect hermaphroditism has much to do with physiological causes. Doctor Stout, of the New York Botanical Garden, who kindly consented to

criticize this paper, pointed out that the bud which was inserted in the Native Red Single might have been a bud mutation. He suggested a repetition of the budding, using many buds of the Prisoner variety, and checking the observation on the effect of budding on the Prisoner flowers. The results of this experiment will be reported in a future paper.

Finally, I wish to point out the possibility that the effect of the operation reported in this paper (of rendering receptive stigmas that are otherwise nonreceptive) belongs to the class of phenomena known as sex change or, better still, change of sex expression, due to mutilation; as, for example, the production of hermaphrodite flowers in a so-called "male" papaya following decapitation, or the conversion of sterile banana flowers into fruit-bearing, though seedless, individuals following an accidental mutilation at the top of the false stem of the plant.⁴

The results of the present observations would indicate another way of obtaining fruit from male papayas; that is, by budding, or grafting buds of the male plant on more vigorous individuals. The papaya is known to lend itself to grafting and other methods of asexual propagation.

⁴ Mendiola, N. B., Effect on banana fruit of premature appearance of the inflorescence, Philip. Agr. 10 (1922) 299-300.

ILLUSTRATIONS

PLATE 1

- FIG. 1. A typical flower of *Hibiscus*, variety Prisoner, not showing the stigmas inside the staminal column.
2. Terminal half of staminal column split, exposing the hidden stigmas.
3. Appearance of infertile ovary of a typical flower at the time of the premature fall of the ovary.
- FIGS. 4 to 8. Different steps in the operation of the staminal column of a bud, twenty-four hours before opening, to expose the stigmas; 4, typical bud twenty-four hours before opening; 5, top of corolla removed with a pair of small scissors; 6, anthers of the part exposed have been removed; 7, point of staminal column over stigma has been cut off and walls of remaining top of column bent aside to expose the stigmas, enlarged; 8, appearance of stigmas twenty-four hours after operation. At this stage the stigmas are functional with cross-fertile pollen.
- 9 to 11. Details of flowers of *H. rosa-sinensis*, var. Woodrow Wilson; 9, normal stigmas of a typical flower; 10, an abnormal flower from which the petals were cut to show the staminal tube. This was split open to show the much-delayed pistil. The stigmas were several centimeters below the top of the tube at the time of blooming; 11, similar to fig. 10, except that the stigmas are nearer to but still a few centimeters below the top of the tube.
- 12 to 14. Details of flower of *H. rosa-sinensis*, var. Two-Days; 12, a typical flower of the variety, showing exposed, normal stigmas; 13, part of a staminal tube, inside of which is the pistil which failed to come out; 14, similar to fig. 13, except that the stigmas came out by breaking through the walls of the tube instead of normally appearing above the pore of the tube.

PLATE 2

- FIGS. 1 to 5. Flower buds of variety Prisoner showing different kinds and degrees of attacks of insects. In fig. 5 the top of a stigma, exposed on account of the attack, is barely visible.
- FIG. 6. A clearer case of the condition shown in fig. 5.
- FIGS. 7 to 13. Similar to figs. 1 to 6, but relating to different varieties, as follows: 7, Unknown-3; 8, Unknown-4; 9, Unknown-3; 10, (Deep Pink × Hawaiian Salmon)-4; 11, Unknown-12; 12, Hawaiian Pink; 13, Unknown-6.

PLATE 3

FIGS. 1 to 11. Styles of different flowers of Prisoner growing on their own roots, showing variability in distance between stigmas and opening of the staminal tube. By referring to Table 1, the frequency distribution of each of the distances shown may be learned.

12 to 21. Similar to figs. 1 to 11, except that the styles shown were from a budded plant. (See also Table 1 for frequency distribution of different types.)

PLATE 4

FIG. 1. *Ornithoptera nephereus* Gray in the act of pollination.

2. Underside of one of the hind wings showing pollen grains on the portion which comes in contact with pollen and stigmas.

3. Ventral side of abdomen showing pollen grains.

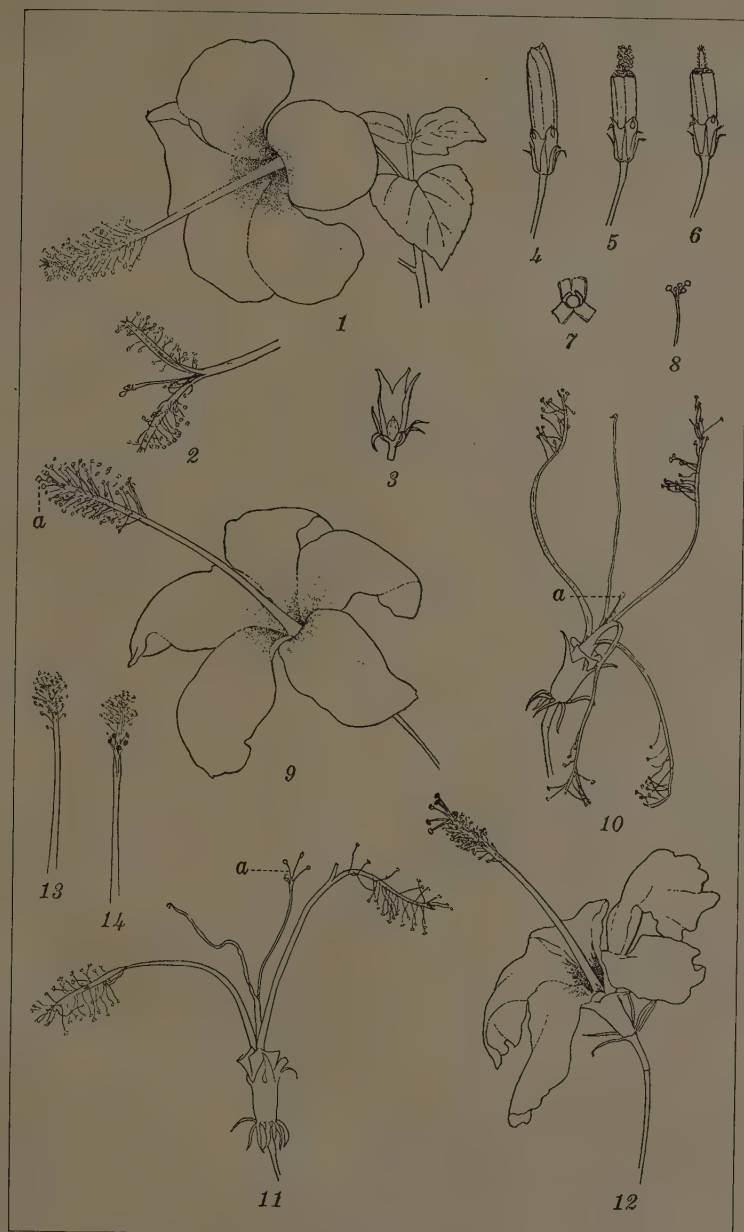


PLATE 1.

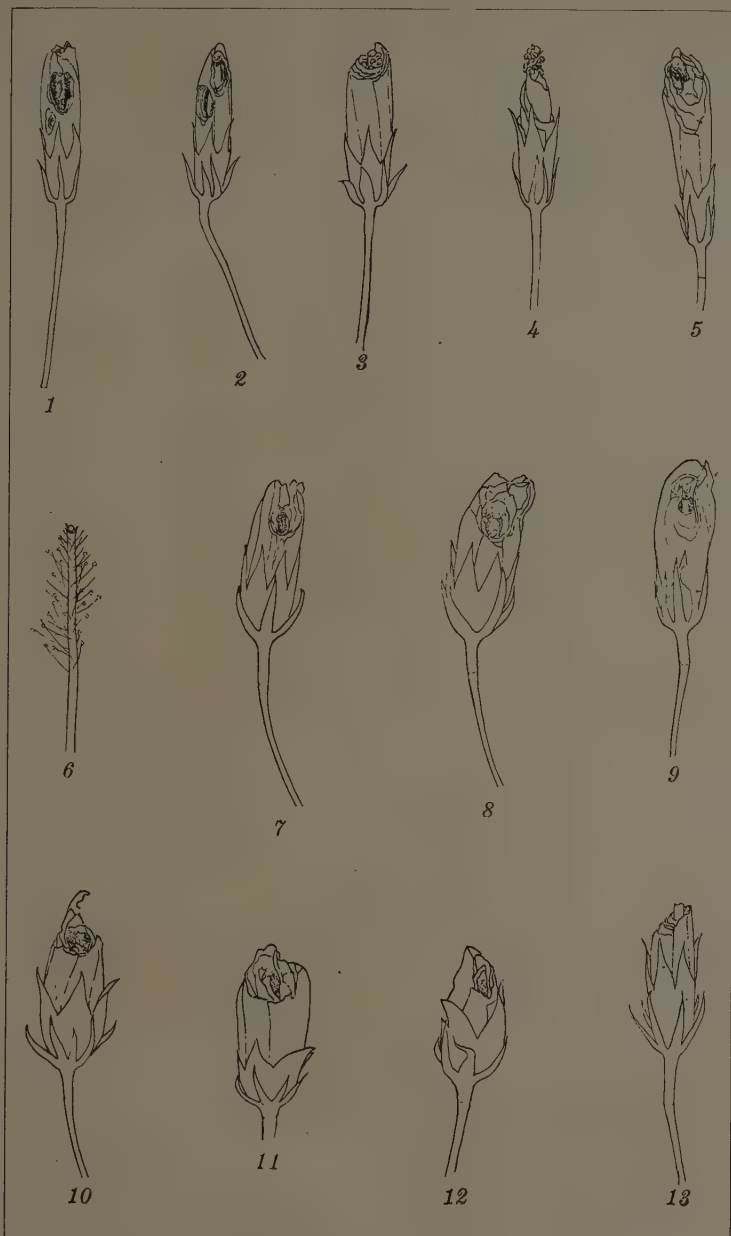


PLATE 2.



PLATE 3.



PLATE 4.

DETERIORATION OF ABACA (MANILA HEMP) FIBER THROUGH MOLD ACTION

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TEN PLATES AND TWO TEXT FIGURES

INTRODUCTION

HISTORICAL

Defective Manila hemp was first noted in 1902, when large quantities of the material reached the United States. The American expert who investigated the trouble attributed it to long storage of the fiber.

After that time, unsound fiber did not attract any particular attention until the latter part of 1920, when complaints from some hemp dealers were received. However, proper consideration was not given the matter until the early months of 1921, after the English importers had received many more shipments of similarly defective material.

Inquiries and observations were then made at the local warehouses where Manila hemp is stored, where it was learned, first, that defective fibers are in many cases found in low grades, such as those from J to DM; second, that they very seldom occur in higher grades, such as those from A to H; third, that defective fibers come from nearly all abacá-growing provinces and are not confined to any specific locality, although the majority of complaints have been against fiber coming from the Bicol region; fourth, that because of the sudden drop in the price of commodities after the World War, enormous quantities of fiber were kept in storage in the local warehouses for from one to two years, or even longer, with the result that in the latter part of 1920 defective Manila hemp became so abundant as to attract the attention of the English importers; and fifth, that sometimes fiber samples that are apparently normal and strong when shipped from the Islands are found weakened and damaged when received in London.

Since then samples of unsound fiber have been sent in by English importers, local fiber dealers, Government fiber inspectors, and many others, requesting information and comment on the causes of the trouble.

In collaboration with Mr. H. Atherton Lee, then mycologist of the Bureau of Science, the preliminary experiments leading to the preparation of this paper were worked out. Various organisms, such as *Aspergillus flavus* Link, *Aspergillus niger* van Tieghem, *Penicillium glaucum* (Link?) Brefeld, *Rhizopus nigricans* Ehrenberg, *Leptosphaeria* sp., one sterile fungus, and four kinds of bacteria were isolated from the diseased fibers. The check cultures from normal fiber yielded, to a very much less degree, the same microorganisms, except *Leptosphaeria* and the sterile fungus.

In order to test the action of these organisms on sound fibers, test tube cultures of moist Manila hemp (grades H and J), absorbent cotton, and filter paper were prepared and inoculated after steam sterilization. After the tubes had been kept two months at room temperature (27 to 30° C.) it was found that *Aspergillus flavus*, *A. niger*, *Leptosphaeria* sp., and the sterile fungus had caused deterioration to about equal extent in Manila hemp and filter paper, but to a very much less extent in the absorbent cotton. The deterioration was so great that the weakened condition could be detected by the hand test alone.

During the progress of the preliminary experiments the results of English investigations on the same subject were noted from time to time, in either published articles or unpublished manuscripts. Some of these results are cited and discussed in this report.

PRESENT WORK

The objects of the present work were to determine the effect of molds on the fiber and to formulate, if possible, practical methods of control.

DESCRIPTION OF DETERIORATED FIBER

Defective fiber is characterized by weakness, brittleness, sometimes a dark color, and a musty odor which is stronger in moist fiber than in dry. Irrespective of grade, it loses the luster natural to normal, well-cleaned fiber. When badly damaged and dry, it is so weak and brittle that it can easily be crushed into a powdery mass in the hand; but when the damage is less, the de-

terioration in tensile strength may be so slight as to escape detection by the hand test alone, in which case the loss in strength can be determined only by machine testing.

As a general rule, the color reveals its actual condition; that is, in almost all cases in the same class of fibers the light-colored hanks are sound and the dark-colored ones are damaged.

Although in the majority of cases defective fiber is darker than normal, color cannot always be considered as definitely associated with unsoundness, for examination of hundreds of samples of supposedly damaged material in these grades has shown that light and dark-colored fibers in the same hank may be equally deteriorated by various organisms, or the dark-colored fiber may even be normal and the light-colored deteriorated; in the latter cases dark-colored fiber (streaky) was found which was not infected at all, but had a natural color resulting from the dark spottings or patches¹ generally present on the outer part of the outer leaf sheaths of the abacá plant, and the light-colored fiber was infected with molds alone. (See Plate 10.) Owing to the presence of sap (possibly containing tannin) which remains after improper drying of the fibers just after stripping, they may have a buff color instead of being white, and still be not in the least inferior in so far as the tensile strength is concerned. In such case color cannot, therefore, be hastily taken as a sure sign of deterioration, though damaged fiber does have a certain dull and dirty shade, a characteristic which will not be passed unnoticed by a keen-eyed, experienced fiber man. Next to lower tensile strength and harder texture, a dull, dirty, dark color, therefore, constitutes another criterion for deciding that a given fiber has deteriorated.

It is interesting to note in this connection that deterioration is not always general throughout the entire length of the fiber, but that a single strand may be defective in the middle and sound at the base and tip, and vice versa.

CAUSAL ORGANISMS

ISOLATION TECHNIC

Using potato glucose agar +1 and beef bouillon +1 as culture media numerous isolations of the organisms present on

¹ In some varieties, as in what is called "abacang bayan," in San Antonio, Loños, Laguna, such patches are natural while in others they are the result of pathological infections, as in the case of *Marasmius*.

stored fibers were made from material obtained from local warehouses in which fibers from all abacá-producing provinces are held for some time before they are shipped abroad.

From damaged fiber, grade J, sent in by Smith, Bell and Company, Limited, and other local abacá dealers, strands were cut into sections of about 1 centimeter long which, after having been moistened with tap water to allow immediate and uniform action of the disinfectant, were soaked in a 1:1,000 solution of mercuric bichloride for one minute and then washed four times in sterile water, after which they were picked up one by one with sterile forceps, plated in five plantings onto each of ten Petri dishes of potato glucose agar +1, and one planting in each of ten bouillon tubes. The cultures were then set aside and incubated at room temperature (27 to 30° C.).

After twenty-four hours it was found that all of the bouillon tubes were clouded, indicating that microorganisms had already started to develop, and thirty of the plate cultures had produced bacterial colonies, but no fungus. From these bacterial cultures dilution plates were made on potato glucose agar +1. On the following day the dilution plates gave rise to three types of bacterial colonies—one grayish, one creamy buff, and one wax brown, the grayish predominating—while twenty-five plantings on the original plates showed fungal growth; transfers were made from the last-named, as well as from the bacterial species, to be used for further studies. A detailed study of the fungal cultures revealed the presence of *Aspergillus flavus*, *A. fumigatus*, *A. niger*, and *Chaetomium funicolum*.

A second series of tests, using the same strength of mercuric bichloride but increasing the interval of soaking from 1 minute to 2, 3, 4, and 5 minutes, respectively, was then made. After forty-eight hours of incubation the first set gave 40 per cent positive results; the second, 25 per cent; the third, 5 per cent; and the fourth was entirely negative. The fungi obtained included all the species isolated from the first series. Repetition of the experiment gave practically the same results, with the bacterial colonies appearing first.

In a subsequent series of isolation experiments the method of eliminating external organisms employed by Altson (unpublished report) was tried. The 1-centimeter sections of damaged fiber were subjected to prolonged washing in running tap water,

followed by rinsing and washing in several changes of sterile water, then plated one by one on plates of potato glucose agar +1, five plantings to each. The experiment was duplicated, using sections from sound fiber of the same grade for check. As was expected, the types of microorganisms isolated from the damaged fiber were greatly increased in number, while from the sound material, curiously, practically the same ones were obtained, although to a very much less degree, the tests being not more than 20 per cent positive. Repetition of the experiment, using samples from different warehouses, gave almost the same results. In the majority of cases bacteria appeared in association with the molds.

The following is a list of the organisms,² excluding bacteria, that were isolated more or less uniformly from numerous samples of disintegrated fiber, mostly grade J, *Aspergillus flavus*, *A. fumigatus*, and *A. niger* being the most prevalent:

<i>Aspergillus flavus</i> Link.	<i>Chaetomium elatum</i> Kunze.
<i>Aspergillus fumigatus</i> Fresenius.	<i>Chlamydomucor racemosus</i> Brefeld.
<i>Aspergillus glaucus</i> Link.	<i>Penicillium glaucum</i> (Link?) Brefeld.
<i>Aspergillus niger</i> van Tieghem.	<i>Rhizopus nigricans</i> Ehrenberg.
<i>Aspergillus wentii</i> Wehmer.	<i>Rhizopus oryzae</i> Went.
<i>Chaetomium funiculum</i> Cke.	Yeast, black (possibly <i>Torula nigra</i> Guill.).
<i>Chaetomium olivaceum</i> C. & E.	Yeast, red (possibly <i>Torula</i>).
<i>Chaetomium olivaceum</i> var. <i>chartarum</i> Ehrenberg.	

Aside from the species listed above two kinds of sterile fungi and *Alternaria* sp. were isolated also, but these were discarded for lack of frequent association with deteriorated fibers.

INOCULATION EXPERIMENTS

The above organisms were inoculated on abacá fiber to ascertain their relations to the weakness of the fiber.

As it was believed that moisture plays an important part in the destruction of the fibers, two sets of inoculation tests were carried out, using dry fiber in the first and moist fiber in the second.

²These were identified by comparing their growth characteristics with various published descriptions, particularly those given in Lafar's Technical Mycology and Ellis and Everhart's North American Pyrenomycetes.

Methods and technic.—Strands, 50 centimeters long, were cut from a hank of sound abacá fiber, grade J. These were divided into eighty-five uniform samples of about 2.5 grams, each of which was folded twice, put into an ordinary test tube provided with a cotton plug, and then sterilized in the autoclave for one hour at 102° C. That this was sufficient for sterilization was indicated by negative results secured on culturing ten of the samples.

While the sterility of the specimens was being tested, their moisture content was determined by taking the samples in the last five test tubes, weighing separately, and then drying to constant weights at 102° C. in an oven. The results show that the fiber samples under experimentation contained on the average 11.7 per cent moisture.

TABLE 1.—Effect of molds on air-dry, sterile abacá fiber, grade J1.

Tube No.	Inoculum.	Color. ^a	Average tensile strength. ^b	Deterioration.
				Per cent.
1 to 4.....	<i>Aspergillus flavus</i>	Light buff	28.4	23.3
5 to 8.....	<i>Aspergillus fumigatus</i>	do.....	26.2	29.2
9 to 12.....	<i>Aspergillus glaucus</i>	do.....	37.7
13 to 16.....	<i>Aspergillus niger</i>	do.....	31.1	16.0
17 to 20.....	<i>Aspergillus ventii</i>	do.....	38.7
21 to 24.....	<i>Chaetomium elatum</i>	do.....	32.9	11.0
25 to 28.....	<i>Chaetomium funiculum</i>	do.....	31.3	15.4
29 to 32.....	<i>Chaetomium olivaceum</i>	do.....	35.0	5.4
33 to 36.....	<i>Chaetomium olivaceum</i> var. <i>charitarum</i>	do.....	36.3	1.8
37 to 40.....	<i>Chlamydomucor racemosus</i>	do.....	42.0
41 to 44.....	<i>Penicillium glaucum</i>	do.....	37.8
45 to 48.....	<i>Rhizopus nigricans</i>	do.....	38.0
49 to 52.....	<i>Rhizopus oryzae</i>	do.....	39.1
53 to 56.....	Yeast, black	do.....	40.3
57 to 60.....	Yeast, red	do.....	39.7
61 to 80.....	Control (check)	do.....	37.0

^a The colors indicated here and elsewhere in this paper are those of Ridgway's Color Standards and Color Nomenclature. Washington (1912).

^b Average breaking strain in kilograms per gram meter of twenty samples.

The first series of inoculations of sterile fiber samples was carried out on August 1, 1923. With the pure cultures of the fifteen kinds of organisms obtained from the isolation experiments aforementioned, infection was artificially made in all of the remaining eighty sterile fiber samples, except the last twenty which were kept as check. There were, therefore, five

test tubes of fiber to each kind of organism—four infected and one sterile, as check. The infection was done by transferring with a sterile platinum needle bits of the mycelial threads, or masses of spores as the case may be, of each individual culture into the fiber samples in test tubes. As soon as the needle was infected by touching the fresh culture, it was thrust many times into the fiber strands in the test tubes, so as to make the infection of the fibers as nearly uniform and as thorough as possible. The entire set was then set aside and allowed to incubate under ordinary room conditions with a temperature ranging from 27 to 30° C.

TABLE 2.—Effect of molds on moist, sterile abacá fiber, grade J1.

Tube No.	Inoculum.	Color.	Average tensile strength.*	Deterioration.
				Per cent.
1 to 4....	<i>Aspergillus flavus</i>	Warm buff with patches of mouse gray.	21.6	36.2
5 to 8....	<i>Aspergillus fumigatus</i>	do.....	11.5	66.0
9 to 12....	<i>Aspergillus glaucus</i>	do.....	27.2	20.0
13 to 16....	<i>Aspergillus niger</i>	do.....	21.9	35.3
17 to 20....	<i>Aspergillus wentii</i>	do.....	28.0	14.6
21 to 24....	<i>Chaetomium elatum</i>	Vinaceous buff.....	21.9	35.5
25 to 28....	<i>Chaetomium funicolum</i>	Colonial buff.....	16.2	52.2
29 to 32....	<i>Chaetomium olivaceum</i>	Deep olive buff.....	18.8	44.7
33 to 36....	<i>Chaetomium olivaceum</i> var. <i>chariarum</i>	do.....	19.9	41.3
37 to 40....	<i>Chlamydomucor racemosus</i>	Warm buff.....	22.9	32.4
41 to 44....	<i>Penicillium glaucum</i>	do.....	24.8	27.0
45 to 48....	<i>Rhizopus nigricans</i>	do.....	36.3	-----
49 to 52....	<i>Rhizopus oryzae</i>	do.....	36.3	-----
53 to 56....	Yeast, black.....	Slate gray.....	35.7	-----
57 to 60....	Yeast, red.....	Livid pink.....	39.3	-----
61 to 80....	Control (check).....	Cream buff.....	34.0	-----

* Average breaking strain in kilograms per gram meter of twenty samples.

Subsequently, another series of inoculations was started, duplicating the first in every respect except in the moisture content of the fibers. In the first series (Table 1) air-dry fiber with a moisture content of only 11 to 12 per cent was used, whereas in the second (Table 2) wet fiber was used; the latter was prepared by adding with a sterile pipette as many cubic centimeters of sterile water as there were grams of the fiber

samples. Two months later (October 1, 1923) the necessary observations were made, taking the dry series first.

Results.—In color no appreciable difference was noted in the dry series between the inoculated and the control; it was only in the texture that the two differed; that is, those inoculated with *Aspergillus flavus*, *A. fumigatus*, *A. niger*, *Chaetomium elatum*, *C. funiculum*, and *C. olivaceum* were not so soft as were the control and the rest of the samples inoculated with other organisms. Neither did the hand tests reveal any difference in the tensile strength of the samples, a condition which favored the belief that none of the organisms used could appreciably damage artificially infected, sterile, dry fiber. Machine tests made on the Louis Shopper machine, however, indicated that with the first two organisms, at least, the deterioration was significant. With the remaining organisms the results given apparently lie within the limits of experimental error.

On the other hand, the deteriorative effect of the molds was much more readily observed on the moist fiber, both in tensile strength and in color (Table 2). The inoculated fibers were darker than the control, although not all the dark-colored inoculated ones were invariably weaker than the light-colored. Again, attention is called to the fact that dark color is not always correlated with unsoundness or a damaged condition of the fiber. The testing data indicate that at least ten of the organisms may cause considerable or marked deterioration in moist fiber.

REISOLATION AND IDENTIFICATION

From the inoculation experiments that gave positive results reisolutions were made on potato glucose agar +1. These reisolutions yielded the organisms originally used.

VARIOUS BELIEFS AS TO THE CAUSE OR CAUSES OF DAMAGE, AND EXPERIMENTS THEREON

COMPARATIVE EFFECTS OF MOLDS ON ABACÁ AND CANTON FIBERS

It has been suggested that adulteration of abacá fiber with other kinds of fiber, such as canton and pacol, might lead to the appearance of a considerable amount of damage, the idea being that canton might deteriorate more readily than abacá. This assumption was incidentally favored by the fact that fibers

of both canton and pacol are by nature weaker than abacá fiber and are also produced in the Bicol provinces whence a large amount of unsound abacá fiber has come. To test the comparative effect of molds in causing deterioration of abacá and canton, another series of inoculations was conducted, using both completely moist abacá and canton fibers of the same grade (F1) as media and the pure cultures of all the organisms proved to cause deterioration as inocula. Care was exercised to make the samples as nearly uniform as possible and to subject them to the same conditions and treatment. With the controls the inoculated specimens were set aside to incubate at room temperature (27 to 30° C.). Forty days later observations were made, as in the previous experiments, taking the tensile strength of the fiber on the machine. The results are given in Table 3.

In this experiment there is no indication that molds cause greater deterioration in canton than in abacá; in fact, in this particular case canton showed somewhat less deterioration than did abacá. Since the test was carried out only on one sample of each product, however, the results should not be considered by any means conclusive, for different results might be obtained from plants from different localities under other conditions.

TABLE 3.—*Comparative tensile strength of abacá and canton fibers affected by molds.*

Tube No.	Inoculum.	Average tensile strength.*	
		Abacá.	Canton.
1 to 5	<i>Aspergillus flavus</i>	19.4	18.9
6 to 10	<i>Aspergillus fumigatus</i>	16.6	12.9
11 to 15	<i>Aspergillus glaucus</i>	25.9	22.7
16 to 20	<i>Aspergillus niger</i>	26.8	20.7
21 to 25	<i>Aspergillus wentii</i>	27.1	17.3
26 to 30	<i>Chaetomium elatum</i>	23.9	24.9
31 to 35	<i>Chaetomium funicolum</i>	14.9	11.6
36 to 40	<i>Chaetomium olivaceum</i>	22.9	13.4
41 to 45	<i>Chaetomium olivaceum</i> var. <i>charitarum</i>	23.7	19.1
46 to 50	<i>Chlamydomucor racemosus</i>	34.4	24.4
51 to 55	<i>Penicillium glaucum</i>	31.6	23.3
Mean of the control		39.35±0.6	29.3 ±0.4
Mean of the infected fiber		23.9 ±1.2	18.5 ±0.4
Difference of the means		15.4 ±1.4	10.9 ±0.6
Per cent of deterioration		39.1	37.2

* Average breaking strain in kilograms per gram meter of twenty samples.

ADMIXTURES OF FIBERS FROM INNER AND OUTER SHEATHS

Harvey-Gibson,⁽⁴⁾ in reporting on the findings from his experimentation, expressed the belief that the defective nature of Manila hemp is partly due to (a) admixture of vascular bundles and parenchyma, which would not be present if only the outside part of the petiole were used, and (b) admixture of fiber stripped from the less mature leaf sheaths from the innermost part of the pseudostem with that from the more mature outer sheaths.

While it appears true from tests made by both Espino⁽³⁾ and myself that fibers from the middle portion of the leaf sheath are weaker than are those from the sides of the same leaf sheath, and that fibers from the innermost leaf sheath are weaker than are those from either the intermediate or the outermost leaf sheaths, these weaker fibers cannot be classified as damaged fibers, in as much as they compare favorably in tensile strength with normal, well-cleaned fiber. Apparently the fibers from intermediate leaf sheaths are also stronger than those from the outermost or innermost sheaths as indicated by the work of Espino⁽³⁾ and by a number of tests made by myself on a single plant. This comparative weakening has been ascribed by various authors to the presence on fibers from the outermost leaf sheaths of "peculiar, thick, strongly silicified plates, known as stegmata,"⁽⁵⁾ and to a less-developed condition of the fibers in the innermost sheaths.

DETERIORATION OF WELL-CLEANED FIBER FROM DIFFERENT LEAF SHEATHS

In order to determine whether the age of the fiber has any relation to the rate of deterioration caused by cellulose-digesting organisms the following experiment was carried out.

An immature nonfructed but well-developed stalk of the Sinibuyas variety of abacá was cut, and the fibers from each leaf sheath were stripped separately with a nonserrated knife. The fibers were numbered from 1 to 18 inward, from the outermost (oldest) to the innermost (youngest) sheath. From each group, three samples, each 0.5 meter long and weighing about 2.5 grams, were cut, coiled into a ring with a diameter of about 1 decimeter, placed in Petri dishes, completely moistened with tap water, and then sterilized for one hour in an oven at about

102° C. Two of the three plates of each of the eighteen groups were then inoculated on September 13, 1924, with spores of *Aspergillus fumigatus* in pure culture and, with the triplicates as check, incubated in an oven with a temperature ranging from 38 to 40° C.

Thirty days later, the necessary observations were made. From each sheath ten representative samples of the inoculated, as well as of the control, were prepared, weighed separately, the tensile strength of each tested on the machine, and the percentage of stretch per 20 centimeters distance noted. The results are given in Table 4.

TABLE 4.—Comparative rate of deterioration by *Aspergillus fumigatus* of well-cleaned fibers from different sheaths of a single plant.^a

Sheath, numbered from outermost.	Average tensile strength. ^b		Average stretch. ^c		Deterioration.		Mean deterioration of the six outermost, six middle, and six innermost leaf sheaths.	
	Inocu- lated.	Check.	Inocu- lated.	Check.	Strength.	Stretch.	Strength.	Stretch.
			P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1.....	36.9	41.4	2.7	3.4	10.8	20.9		
2.....	29.4	36.2	1.6	2.1	18.8	23.1		
3.....	30.2	44.3	1.7	2.1	31.9	18.1		
4.....	34.7	50.2	2.2	2.9	30.9	25.3		
5.....	30.8	47.6	2.2	3.0	35.4	26.6		
6.....	31.8	42.3	2.0	2.6	24.8	23.5	24.7±1.4	22.2±0.8
7.....	32.7	39.1	2.2	3.1	16.3	26.5		
8.....	30.1	49.6	2.2	2.8	39.2	22.0		
9.....	36.9	45.1	2.1	2.5	18.3	18.1		
10.....	30.5	41.7	2.1	2.6	26.8	18.8		
11.....	34.5	52.2	2.0	2.9	33.9	30.2		
12.....	32.9	46.3	2.1	2.8	29.0	26.4	27.2±1.8	23.8±1.0
13.....	35.1	45.5	2.2	3.0	22.9	25.8		
14.....	33.8	43.8	2.1	2.6	22.9	21.1		
15.....	32.5	50.7	2.1	2.8	35.3	25.6		
16.....	32.1	50.5	2.0	2.8	36.5	26.4		
17.....	30.3	49.1	1.9	2.8	38.2	32.1		
18.....	30.2	45.4	2.3	3.0	33.5	22.7	31.3±1.6	25.5±0.6
Mean difference between first and second groups.....							2.5±2.3	1.6±1.4
Mean difference between second and third groups.....							4.2±2.4	1.7±1.3
Mean difference between first and third groups.....							6.7±2.2	3.3±1.1

^a This plant being immature, the innermost fibers are naturally not so developed as are the outermost.

^b Average breaking strain in kilograms per gram meter of twenty samples.

^c Average percentage of stretch of the fiber per 20-centimeter distance of the sample.

DETERIORATION OF POORLY CLEANED FIBER

It has been the opinion among fiber men who are more or less conversant with the subject that only fibers of the poorly cleaned grades deteriorate; that is, fibers of excellent or good cleaning ranging from grade A to grade S are thought to be immune, while partially cleaned fibers, from grade I down, are susceptible. That this view should be modified is indicated by the considerable losses sustained in well-cleaned fiber in the tests reported above, as well as by observations made on deterioration under commercial conditions.

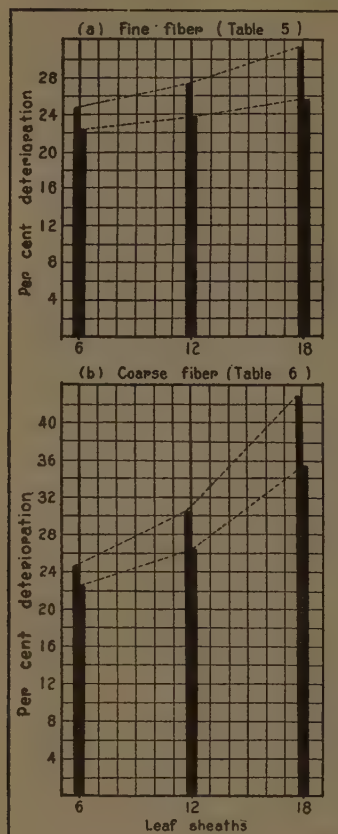


FIG. 1. Showing graphically the rôles that age and poor or partial cleaning of fiber play in relation to deterioration. Left column, percentage of deterioration in strength; right column, percentage of deterioration in stretch.

employed, fiber of a better quality is produced. Generally the strippers in these localities classify their fiber into the following

That commercial practices in the production of Manila hemp play an important part in its subsequent deterioration, however, is unquestioned. In stripping the fiber two kinds of knives are used, the serrated and the nonserrated. With the serrated knife lower grades, such as from Good fair to Coarse brown and down, are produced, while with the nonserrated knife the higher grades, ranging from Tagal braid to Brown, are obtained. In Batangas, Cavite, Laguna, and Tayabas Provinces, where the nonserrated knife is

three grades, which conform in principle with the Fiber Standardization Board's standard:

Sheath.	Grading.
1 to 6 (outermost)	Third class (S1-H).
7 to 12 (intermediate)	Second class (C-E).
13 to 18 (innermost)	First class (A-B).

According to this grading, fibers of the First class are the youngest; fibers of the Second class, the intermediate; and fibers of the Third class, the oldest; but, when cleaning is poor, or only partial, the fibers are classified as fair, medium, and coarse.

On the other hand, in the Bicol and Visayan provinces as well as in Mindanao, where both kinds of stripping knives are used (but usually the serrated), the strippers do not separate the fibers into grades as they do in Luzon, but mix them together and leave them for the middlemen or consumers to classify. Following the standard adopted by the Fiber Standardization Board these fibers are ultimately graded as A, B, C, etc. (United Kingdom grades), in the case of partially cleaned fibers or fibers stripped with the serrated knife.³

In order to find out whether or not poor or partial cleaning of the fiber favors deterioration another set of inoculation experiments was carried out beginning September 13, 1924, using partially cleaned fiber or fiber stripped with the serrated knife. This experiment was a duplicate of that recorded in Table 4, except that in this case coarser fiber, or fiber stripped with the serrated knife, was used.

After thirty days of incubation in an incubator with a temperature ranging from 38 to 40° C. the necessary observations were taken and the results are shown in Table 5.

The results show that, all things being equal, the coarser fiber sustained somewhat greater loss in deterioration, both in tensile strength and in stretch, when infected with the cellulose-digesting organism than did the finer fiber. This is substantiated by taking the mean percentages of deterioration of the fine fiber (Table 4) which are approximately 27.8 and 23.8, for strength and stretch, respectively, and comparing them with those of the coarse fiber amounting to approximately 32.7 and

³ For reference in grading abacá fiber see Administrative Order No. 44, Bureau of Agriculture, P. I., 1924.

28.0. Similarly, there is shown a distinct tendency for the fibers to deteriorate more rapidly from the outermost to the innermost.

TABLE 5.—Comparative rate of deterioration by *Aspergillus fumigatus* of coarse fibers from different sheaths of a single plant.^a

Sheath, numbered from outermost.	Average tensile strength. ^b		Average stretch. ^c		Deterioration.		Mean deterioration of the six outermost, six middle, and six innermost leaf sheaths.	
	Inocu- lated.	Check.	Inocu- lated.	Check.	Strength.	Stretch.	Strength.	Stretch.
			P. ct.	P. ct.	P. ct.	P. ct.	P. ct.	P. ct.
1.....	29.3	38.9	2.6	3.7	24.6	29.2		
2.....	30.4	42.0	2.5	3.3	27.5	24.9		
3.....	29.3	40.7	2.7	3.7	28.1	27.3		
4.....	29.4	35.1	2.2	2.6	16.2	13.5		
5.....	32.2	45.0	2.0	2.5	26.2	20.6		
6.....	34.5	49.9	2.5	3.2	30.8	22.2	24.7±1.0	22.2±1.4
7.....	36.6	46.3	2.7	3.7	21.2	26.5		
8.....	31.6	50.1	2.2	3.2	36.9	30.0		
9.....	35.6	48.5	2.7	3.5	26.8	22.5		
10.....	31.3	44.5	2.6	3.2	29.6	19.7		
11.....	31.0	44.6	2.4	3.1	30.4	21.8		
12.....	28.3	45.6	2.2	3.6	27.8	40.3	30.5±1.5	26.4±1.7
13.....	25.2	42.6	2.4	3.0	40.9	38.9		
14.....	32.2	54.7	2.4	4.1	41.2	42.1		
15.....	28.7	46.2	1.9	2.8	38.0	30.3		
16.....	27.5	48.3	2.0	3.2	43.2	35.8		
17.....	22.6	53.1	1.7	3.2	57.3	46.0		
18.....	24.5	51.0	2.0	2.8	51.0	30.1	43.0±1.1	35.5±1.1
Mean difference between first and second groups							5.8±1.5	4.2±2.2
Mean difference between second and third groups							12.5±1.8	9.2±2.0
Mean difference between first and third groups							18.3±1.1	13.3±1.8

^a This plant being immature, the innermost fibers are naturally not so well developed as are the outermost.

^b Average breaking strain in kilograms per gram meter of twenty samples.

^c Average percentage of stretch of the fiber per 20-centimeter distance of the sample.

DISEASES OF THE ABACÁ PLANT

The view has been expressed that bunchy-top (root rot) and heart rot, which are serious diseases of the abacá plant in certain districts of the Philippines, may have some connection with deterioration of the fiber. To throw some light on the relative strength of fiber from diseased and healthy plants five plants affected with bunchy-top in varying stages of infection and five healthy plants, all of approximately the same age and from Cavite Province, were examined. The fibers were all stripped in the same manner with the nonserrated knife, those from each plant being mixed, sampled at random, and tested on the

Louis Shopper machine for comparative tensile strength. The results are given in Table 6.

TABLE 6.—*Comparative tensile strength of fibers obtained from normal and from diseased abacá plants affected by bunchy-top.*

Source of material.	Average tensile strength. ^a		Loss due to disease.	Remarks.
	Healthy.	Diseased.		
			<i>P. ct.</i>	
Amadeo.....	46.7	44.8	4.1	Young infection.
Indang.....	50.2	47.0	6.4	Do.
Mendez.....	48.4	43.8	9.5	Old infection.
Silang.....	47.8	41.4	13.3	Very old infection.
Do.....	54.5	48.6	10.6	Do.
Average.....	49.5	45.2		

^a Average breaking strain in kilograms per gram meter of twenty samples.

While the data indicate that fiber from the diseased plants is somewhat weaker than that from healthy ones this cannot be ascribed to the deterioration of the fiber but, rather, to its failure to develop properly under the handicap of diseased conditions.

DETERIORATION UNDER COMMERCIAL METHODS OF STORAGE

In order to determine whether the temperature and moisture conditions in warehouses were suitable for rapid deterioration of stored fiber through fungous action, a comparative test was carried out as follows:

On February 4, 1924, a self-recording hygro-thermograph was placed among the bales of abacá fiber in the central warehouse of Smith, Bell and Company, one in that of Hanson and Orth Company, and another in the Bureau of Science, all located in Manila. Side by side with these hygro-thermographs were set out twelve cotton-plugged test tubes of sterile, wet abacá fiber (grade B), six of which were inoculated with *Aspergillus fumigatus*, and the other six left sterile as control.

Three observations were made during two months of incubation; the first on the twentieth day, the second on the fortieth day, and the third on the sixtieth day, testing on the machine the comparative tensile strength of all the fibers. The results obtained are given in Table 7.

As indicated in Table 7, the deterioration under warehouse conditions was essentially the same as in the laboratory exper-

iment carried on simultaneously at the Bureau of Science, which shows that commercial storage conditions at the time of the test were highly favorable to the development on wet fiber of one of the principal organisms concerned in the destruction of fiber.

TABLE 7.—*Effect of prolonged storage of damp mold-infected fiber under warehouse conditions.*

Location.	Relative humidity.			Relative temperature.			Incubation period.	Average tensile strength.*		Deterioration.
	Minimum.	Maximum.	Average.	Minimum.	Maximum.	Average.		Inoculated.	Control.	
	P. ct.	P. ct.	P. ct.	°C.	°C.	°C.	Days.			P. ct.
Smith, Bell and Company.....	58	92	75.7	22	35	30.4	20	38.7	41.4	6.5
							40	32.6	41.0	20.5
							60	16.2	40.2	59.7
Hanson and Orth Company.....	74	94	83.7	32	31	29.0	20	39.9	42.4	5.7
							40	32.3	41.0	21.1
							60	17.2	41.4	58.5
Bureau of Science	64	82	73	24	30	27.2	20	37.6	40.4	7.1
							40	32.0	41.4	22.8
							60	18.8	40.7	53.8

* Average breaking strain in kilograms per gram meter of twenty samples.

With regard to temperature, it is shown that the maximum that prevailed in the warehouses during the experiment ranged from 30 to 35° C., which, in itself, is not sufficiently high to cause disintegration of the fiber, but is suitable for the development of the cellulose-digesting organism responsible for the deterioration.

Manila hemp, like other vegetable fibers, is exposed to infection by various microorganisms from the time it is stripped from the leaf sheath of the abacá plant. When the fiber is kept in a place where favorable conditions for the growth of these microorganisms prevail, especially when it is not thoroughly dried (as is often the case in places where rain is more or less equally distributed throughout the year), it is likely to be destroyed in the course of a few weeks or months.

EFFECT OF HEAT ON ABACÁ FIBER

The effect of heat on the fiber was also determined, for undoubtedly abacá fiber, like cotton fiber, is likewise affected by this agent. As pointed out by Matthews,⁽⁵⁾ the higher the

temperature to which cotton fiber is subjected, the greater the loss in weight and strength resulting from the process of dehydration of the cellulose, accompanied by structural disintegration of the fiber.

Fourteen uniform samples of abacá fiber (grade B), 0.5 meter long and weighing 2.5 grams each, were prepared in clean Petri dishes. The first group (1 and 2) was kept in the laboratory. All of the other groups were heated in an oven for an hour at the following temperatures: The second (3 and 4) at 60° C.; the third (5 and 6) at 80° C.; the fourth (7 and 8), at 100° C.; the fifth (9 and 10), sixth (11 and 12), seventh (13 and 14), and eighth (15 and 16) at 120° C. The last three were previously moistened with tap water representing 50, 100, and 200 per cent of their respective weights. Each sample was then taken out of the dish to permit air drying of the wet samples and restoral of the atmospheric moisture of the dried samples. The dried samples were observed to be hard and brittle, but they regained their moisture and softness on exposure to the air. Twenty samples were subsequently prepared from each group, the weight of each as air-dry fiber obtained, and the tensile strength as well as the stretch of each tested on the Louis Shopper machine. The results are given in Table 8.

It is apparent from Table 8 that abacá fiber, like cotton fiber, is affected by heat, the effect being noticeable in color, tensile strength, and stretch. The air-dry white sample, having about 11 to 12 per cent moisture when heated for three hours at 60° C. in an oven, did not show any marked difference in color

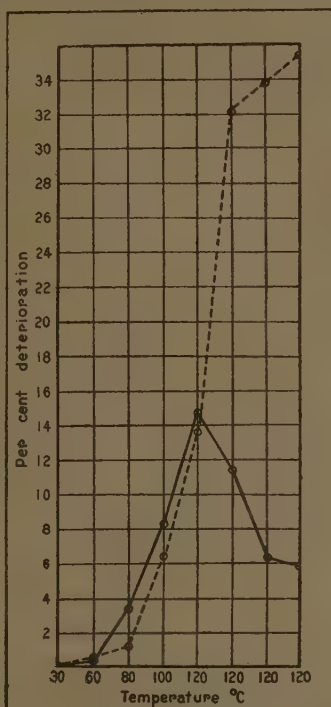


FIG. 2. Graphic illustration of the effect of heat on abacá fiber. Solid line, percentage of deterioration in tensile strength; broken line, percentage of deterioration in stretch.

and sustained no appreciable loss in strength and stretch; the change being very little even at 80° C. At 50 per cent moisture and 120° C., however, the changes became marked in both tensile strength and stretch, the latter increasing as the moisture was raised to 200 per cent. The color changed gradually to light buff and then warm buff as the temperature was raised from 80 to 120° C.

TABLE 8.—Effect of heat on tensile strength and on stretch of air-dry and wet fiber.

Samples.	Moisture when heated.	Temperature.	Color.	Average tensile strength. ^a	Average stretch.	Deterioration.	
						Strength.	Stretch.
	P. ct.	°C.			P. ct.	P. ct.	P. ct.
1 and 2.....	11 to 12	80	White.....	48.6	4.3	(^b)	(^b)
3 and 4.....	11 to 12	60do.....	48.5	4.3	0.3	0.5
5 and 6.....	11 to 12	80do.....	46.9	4.3	3.5	1.2
7 and 8.....	11 to 12	100	Light buff..	44.7	4.1	8.3	6.5
9 and 10.....	11 to 12	120	Buff.....	41.4	3.7	14.8	13.6
11 and 12.....	50	120	Warm buff..	43.0	2.9	11.4	32.1
13 and 14.....	100	120do.....	45.5	2.9	6.4	33.9
15 and 16.....	200	120do.....	45.7	2.8	5.9	35.4

^a Average breaking strain in kilograms per gram meter of twenty samples.

^b Check.

EFFECT OF SALT WATER ON FIBER IN TRANSIT

It is believed by some that occasional wetting of fiber by salt water during transit may be directly responsible for its deteriorated condition on arrival at destination. To test the action of sea water, twelve test tubes of uniform sound abacá fiber (grade B) were prepared. Six of these were moistened with tap water and the rest with sea water before sterilization; four of each group were inoculated with *Aspergillus flavus*, leaving two tubes from each group as control. Observations were made after thirty days of incubation at room temperature (27 to 30° C.) and the comparative tensile strengths of the samples were derived by machine tests. The results are shown in Table 9.

It will be seen from Table 9 that the deterioration of the samples moistened with tap water was much greater than that of the samples moistened with salt water, the saline content of the solution acting as an inhibiting agent on the development of the mold used. Analyses made by Mr. A. S. Argüelles, chief of the division of soils and fertilizers, Bureau of Science, also

show that there is no appreciable difference in the chlorine content of deteriorated and sound fiber. Furthermore, it is a fact that not all deteriorated fiber samples have been wetted with sea water, and therefore deterioration cannot be attributed to the occasional wetting with sea water during transit.

TABLE 9.—*Relation of salt water to deterioration.*

Samples.	Average tensile strength.*		Deterioration.
	Inoculated.	Control.	
Moistened with tap water.....	20.1	45.9	P. cl. 56.3
Moistened with sea water.....	31.1	46.2	32.7
Difference in rate of deterioration.....			23.6

* Average breaking strain in kilograms per gram meter of twenty samples.

COMPARATIVE HISTOLOGICAL STUDIES OF SOUND AND UNSOUND FIBERS

Results of the foregoing experiments clearly demonstrate that deterioration of fiber may be due to the action of the cellulose-digesting organisms. Hence, comparative histological studies on both sound and damaged fibers were undertaken to determine the presence or absence of these organisms.

Sections of abacá fiber (grades D and J), about 5 millimeters long, from both sound and defective bales, were cut and treated separately with 10 per cent solution of hydrofluoric acid. After forty-eight hours the specimens were thoroughly washed in running water, dehydrated, cleared, embedded in paraffine, and sections made with a rotary microtome, 10 microns in thickness. From each of the samples twenty-five good slides were prepared and stained, using different kinds of stain, such as Hiltner's stain, Planeze III B, etc., to bring out clearer differentiation between the organism and the fiber tissues.

As will be noted in Plates 4 and 5, the two fiber samples (sound and defective) differ greatly in two ways; namely, (a) bundles and tubes of the sound sample are of proper shape, whereas the defective sample is much disorganized; and (b) the sound sample shows no fungal infection, whereas the defective sample clearly demonstrates the presence of the fungus filaments embedded in the fiber tissues, marked X. It can be readily seen from this that the mold is devouring all parts of the fiber.

The viscose method, as employed by Cross and Bevan⁽²⁾ and modified by Thaysen and Bunker⁽⁶⁾ in their studies of cotton fiber, was then tried. Five-millimeter sections were cut from both sound and damaged fibers and soaked separately in a mixture of sodium hydrate and carbon bisulphide for from one to two hours, mounted on glass slides with cover slips, and then examined under the microscope.

Of one hundred slides of damaged abacá fiber of grades S1, I, J, and K from the warehouses of Smith, Bell and Company and of Hanson and Orth Company examined, about 80 per cent showed the presence of the cellulose-digesting organisms embedded in the fiber tissues (Plates 5 to 7). It is not surprising to note that some of the supposedly damaged fibers turned out negative when examined, in as much as in nature infection does not always occur throughout the entire length of the individual strand, but more often is limited to parts thereof which may happen to be exposed to the infecting organisms.

On the other hand, examination of normal fiber treated in the same way showed, in the majority of cases, no fungal hyphæ, either on or inside the fiber strands and individual ultimate fiber tubes; that is, of one hundred mounts examined, only five were positive and all the rest negative. Fresh, newly stripped fiber treated similarly showed, when examined, complete absence of any kind of infection.

The viscose method, as modified by Thaysen and Bunker,⁽⁶⁾ is useful in demonstrating the presence of fungal hyphæ in damaged fiber; however, further modification for the purposes of the present work seemed advisable. In determining whether or not molds or other microorganisms are present in the fiber tissues, I found that by soaking the fiber sections in carbon bisulphide for at least six hours (better still overnight) and then transferring them into a 5 per cent sodium hydrate solution, keeping them for another six hours, after which the sections are ready for mounting and examination under the microscope, at least two great advantages are gained; namely, (a) no yellow precipitate or air bubbles are formed in the fibers treated, and (b) the fiber elements are rendered semitransparent and distended but neither curled up nor split into ultimate fiber tubes. This leaves the microorganisms, if there be any at all, intact with their natural form, color, and position unchanged, rendering their detection easier.

MEANS OF CONTROLLING DETERIORATION

Complete elimination of the causal organisms is the surest way of stopping deterioration, but there are many practical difficulties in the way of accomplishing this. The molds that have been found responsible for the damage are by nature omnipresent, so that fiber is doubtless exposed to infection as soon as it is extracted from the leaf sheaths of the abacá plant. Bright and his collaborators(1) have tried various chemicals in connection with their study of mildew in cotton goods, as yet with comparatively meager results. Therefore, the only method of control at present recommended is the adoption of precautions that will reduce the liability to attack. Such precautions should, of course, deal mainly with the elimination of the factors favoring deterioration, which are discussed in the order of their importance in the following paragraphs.

Improper drying of fibers before baling and storage.—Of the different factors favoring deterioration of abacá fiber by the action of cellulose-digesting organisms, particularly the molds of the *Aspergillus* group, abundant moisture appears to be the most important. Therefore, great stress should be laid on the importance of drying all fiber properly before it is baled, stored, or shipped to its final destination. Properly dried fiber should not contain, on an average, more than 11 to 12 per cent of moisture, under Manila conditions. Even with this amount of moisture abacá fiber may lose 10 to 15 per cent of its tensile strength in sixty days by the action of the cellulose-digesting molds; and when the moisture content is raised to 100 per cent, the loss may be three or more times as great.

Keeping too long in storage.—Experiments have shown that moist, fine-grade fibers (grade B), when artificially inoculated with the cellulose-digesting organisms, sustain a loss of about 6 per cent in tensile strength after twenty days, about twenty per cent after forty days, and 55 per cent when incubation is extended to sixty days. For this reason, and because it is impossible to keep fiber from becoming infected with the omnipresent cellulose-digesting organisms, and since conditions obtaining in most of the warehouses are favorable for the development of these organisms, wet fiber should not be stored for long periods.

In the majority of cases speculation is at the root of any protracting of the period of storage and by this means losses

from deterioration are courted, not only from the action of the cellulose-digesting organisms, but also from other causes, such as rats, termites, and possibly fire.

Inadequate ventilation in the warehouses.—Experiments have shown that the cellulose-digesting organisms found to cause deterioration in fiber thrive best where humidity is high, and owing to inadequate ventilation this condition prevails in most of the warehouses where fiber is stored for a time before shipment abroad. Therefore, warehouses should be adequately ventilated.

Lack of care in handling fiber.—To minimize the chances of infection by the cellulose-digesting organisms, the need of proper care in handling fiber from the field to the factory cannot be too strongly emphasized. The sheds and warehouses where drying, sorting, baling, and other minor operations take place should be kept clean and dry, so as to keep the fiber uncontaminated. No refuse of any kind should be allowed to remain on the premises for even a day, but should be burned along with the waste fiber, for it constitutes a veritable hotbed of infection, and neglect in this respect will result in a rich crop of new cases of attack.

SUMMARY

1. This paper deals with abacá-fiber deterioration, numerous complaints against which have been received from fiber dealers of London since the latter part of 1920.

2. Deterioration is not confined to fibers from any one locality, although more cases of damage have been observed in those coming from the Bicol region.

3. Defective fiber is characterized by weakness, brittleness, a dull, dirty, dark color, and a musty odor, which is stronger in moist fiber than in dry.

4. The action of organisms belonging to the cellulose-digesting types like *Aspergillus flavus*, *A. fumigatus*, *A. glaucus*, *A. niger*, *A. wentii*, *Penicillium glaucum*, *Chaetomium elatum*, *C. funiculum*, *C. olivaceum*, and *C. olivaceum* var. *chartarum* will cause deterioration when conditions are favorable for their growth. Sometimes a species of *Alternaria* may be present and also cause damage.

5. There are at least five factors conducive to rapid deterioration of fiber by the cellulose-digesting molds; these are abundant moisture content, poor or partial cleaning, long storage of moist fiber, inadequate ventilation in the warehouses, and

lack of care in handling. Elimination of these factors would probably minimize deterioration due to molds to a point at which it would be practically nil.

ACKNOWLEDGMENTS

I am indebted to Dr. N. G. Teodoro, acting chief of the plant-pests control division, Bureau of Agriculture, for his valuable suggestions during the progress of the experiments; to Mr. A. S. Argüelles, chief of the division of soils and fertilizers, Bureau of Science, for the quantitative analysis of fibers for salt content; to Mr. M. Villaraza, of the fiber division, Bureau of Agriculture, for furnishing some of the materials used in the experiments; and to Messrs. E. Cortes and M. Ligaya, photographer and illustrator, respectively, of the Bureau of Science, for aid in the preparation of the illustrations.

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ILLUSTRATIONS

[All photographic illustrations were made by E. Cortes.]

PLATE 1

- FIG. 1. A "knot" of deteriorated abacá fiber (grade S1) from Camarines Norte. About $\frac{1}{4}$ natural size.
2. Portion of sample shown in fig. 1, showing discolored patches in detail. Note compacted strands which are so weak and brittle that they can be crushed into a powderlike mass in the hand. About $\frac{3}{4}$ natural size.

PLATE 2

- FIG. 1. Abacá fiber (grade S1) inoculated with *Aspergillus fumigatus* after moistening with sea water. The strands were broken into pieces when crushed in the hand. About $\frac{3}{4}$ natural size.
2. As in fig. 1, but not inoculated. Apparently it has not deteriorated, as the strands did not break into pieces when crushed in the hand. About $\frac{3}{4}$ natural size.

PLATE 3

- FIG. 1. Abacá fiber (grade S1) inoculated with *Aspergillus fumigatus* after moistening with tap water. Like that shown in Plate 2, fig. 1, it suffered from deterioration, and to a greater extent. About $\frac{3}{4}$ natural size.
2. As in fig. 1, but not inoculated. Like that shown in Plate 2, fig. 2. It did not sustain any loss in strength. About $\frac{3}{4}$ natural size.

PLATE 4

- FIG. 1. A transverse section of a single strand of sound abacá fiber (grade J) composed of five fiber bundles. Note parenchymatous cells surrounding them. Stained with Bismarck brown. $\times 125$.
2. A single bundle from the strand shown in fig. 1, highly magnified to show ultimate fiber tubes in detail. $\times 500$.

PLATE 5

- FIG. 1. A transverse section of a commercial strand of defective abacá fiber (grade J) composed of five fiber bundles, two of which (\times) are damaged and the rest (+) apparently good. Stained with Bismarck brown. $\times 125$.
2. A portion of strand shown in fig. 1, highly magnified to show much-disorganized cell tissues and fungus filaments bordering the cavity \times . $\times 500$.

PLATE 6

- FIG. 1. A commercial strand of defective abacá fiber (grade S1) treated with the viscose treatment. Note fungus threads (*Aspergillus flavus*) penetrating the entire strand. $\times 125$.
2. A portion of a commercial strand of defective abacá fiber (grade S1) treated with the viscose treatment; highly magnified to show in detail the mode of penetration of the fungus filaments (*Aspergillus fumigatus*). Note peculiar globular swellings and conidiophores. $\times 500$.

PLATE 7

- FIG. 1. Separate bundles of a strand of deteriorated abacá fiber (grade S1) after viscose treatment. Note penetration of the fungus filaments (*Aspergillus niger*) through the lumen (\times). $\times 125$.
2. Ultimate fiber tubes of defective abacá fiber (grade S1) after viscose treatment; highly magnified to show in detail the penetration of the fungal hyphæ (*Aspergillus fumigatus*) through the lumen of the uppermost and lowermost; the middle, which is out of focus, is free from infection. $\times 500$.

PLATE 8

All but fig. 5 are camera lucida drawings of *Aspergillus fumigatus* in different stages of development. (Drawings by Serrano.)

- FIG. 1. Two conidiophores. $\times 300$.
2. Spores. $\times 300$.
3. Spores, germinated. $\times 300$.
4. Hyphæ with peculiar globular swellings, and conidiophores. $\times 300$.
5. A portion of defective strand of abacá fiber (grade J) showing fructification of *Chaetomium funiculum*. $\times 125$.
6. A single immature ascus. $\times 350$.
7. A single mature ascus. $\times 350$.
8. Mature ascospores. $\times 350$.

PLATE 9

Three grades of abacá fiber (from left, B, J, and M), inoculated with different molds causing deterioration, and incubated for thirty days. Note more luxuriant growth of the molds, especially *Chaetomium*, on the coarser grades.

- FIG. 1. With *Aspergillus flavus*. $\times \frac{1}{2}$.
2. With *Aspergillus fumigatus*. $\times \frac{1}{2}$.
3. With *Aspergillus niger*. $\times \frac{1}{2}$.
4. With *Chaetomium funiculum*. $\times \frac{1}{2}$.

PLATE 10

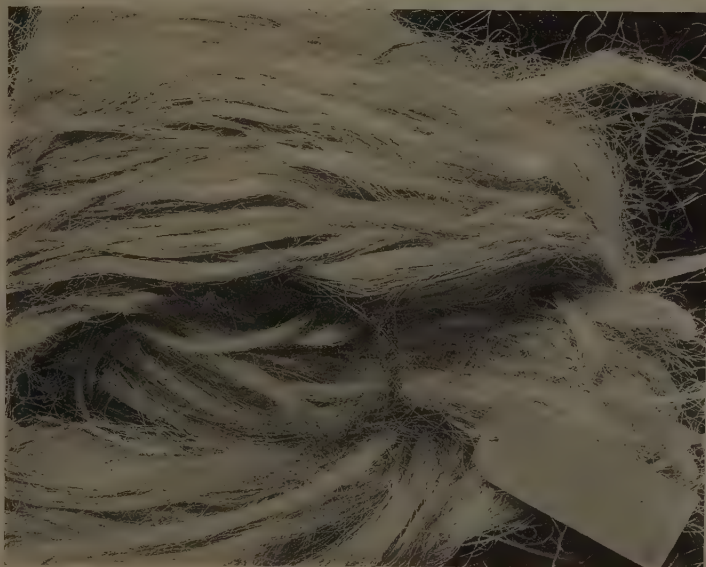
[Water-color drawings by M. Ligaya.]

- FIG. 1. Normal abacá, Streaky No. 1, with a portion showing apricot orange to pansy purple color quite common on fibers from the outermost leaf sheaths.

- FIG. 2. Normal abacá, B, showing ivory white color with the characteristic luster of good fiber.
3. Deteriorated abacá, B, showing cartridge buff to tilleul buff color resulting from infection with molds.
 4. Deteriorated abacá, B, showing cream buff to vinaceous buff color with pale mouse gray patches resulting from infection with bacteria, yeast, and molds.

TEXT FIGURES

- FIG. 1. Showing graphically the rôles that age and poor or partial cleaning of fiber play in relation to deterioration. Left column, percentage of deterioration in strength; right column, percentage of deterioration in stretch.
2. Graphic illustration of the effect of heat on abacá fiber. Solid line, percentage of deterioration in tensile strength; broken line, percentage of deterioration in stretch.

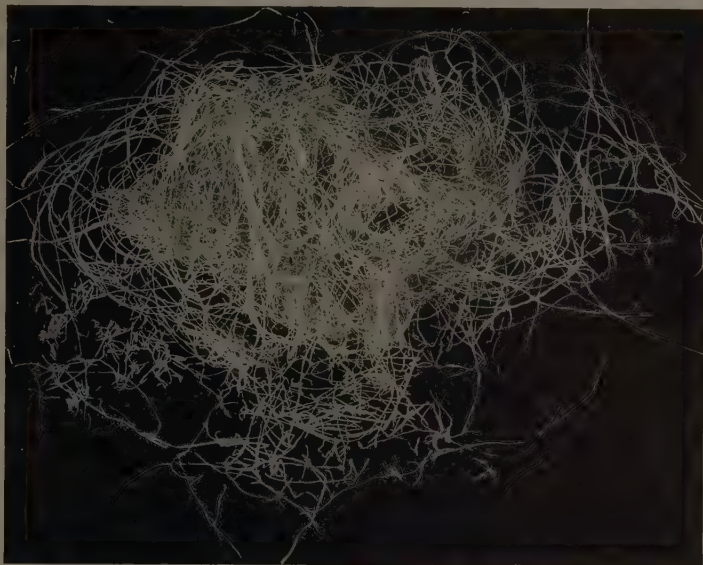


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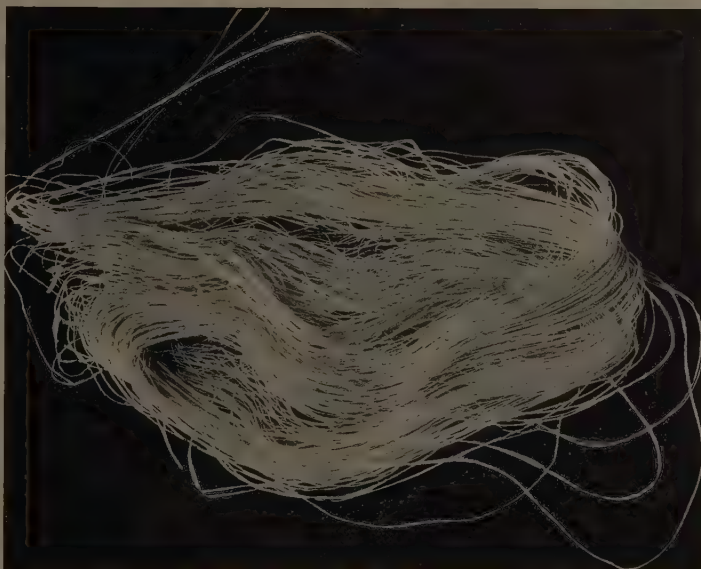


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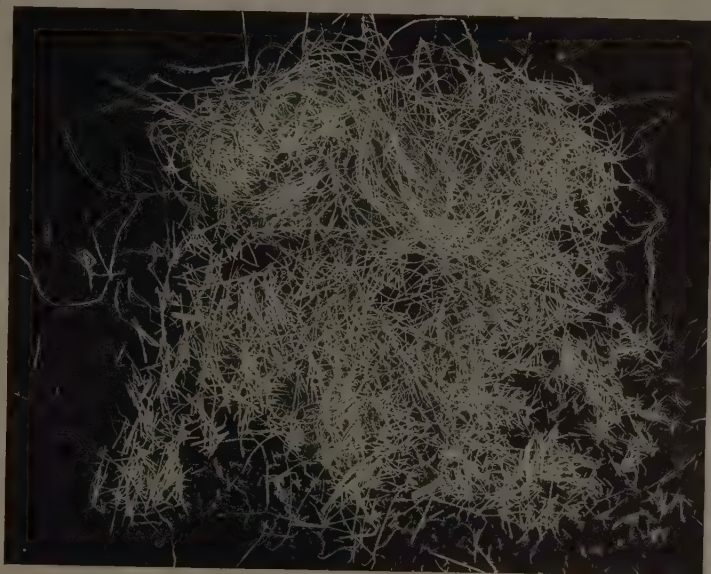
PLATE 1.



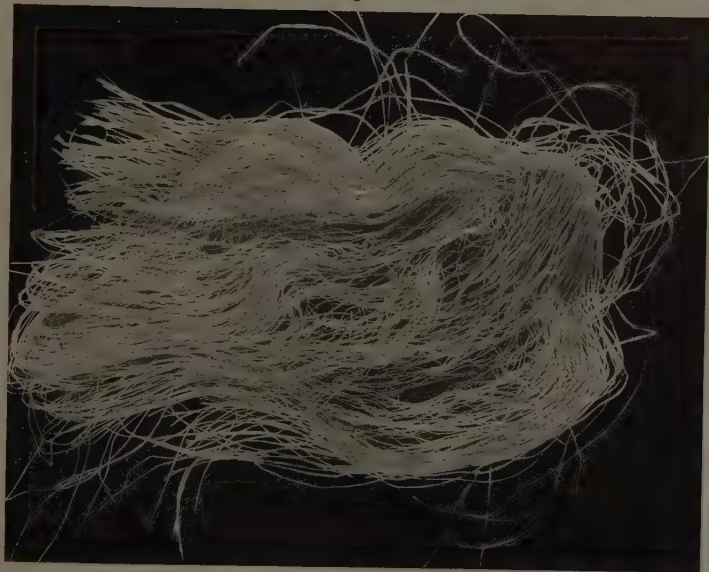
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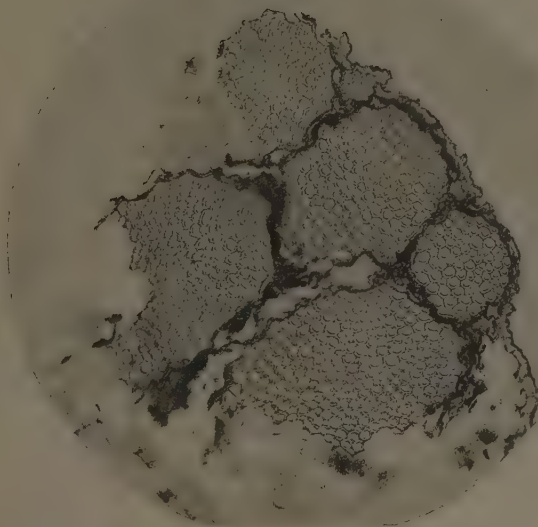
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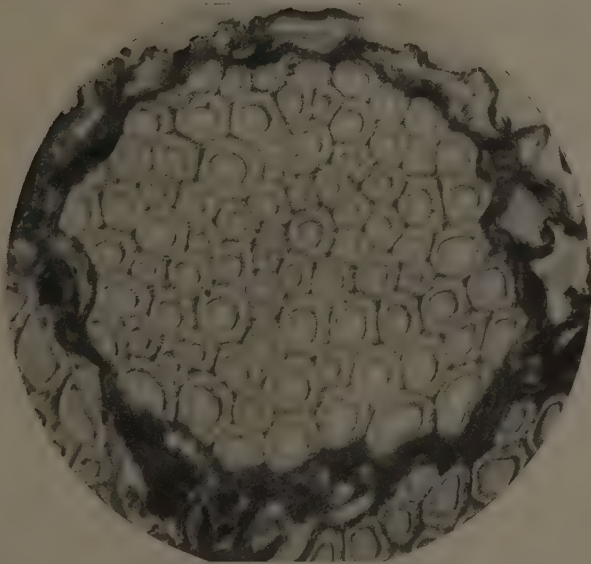
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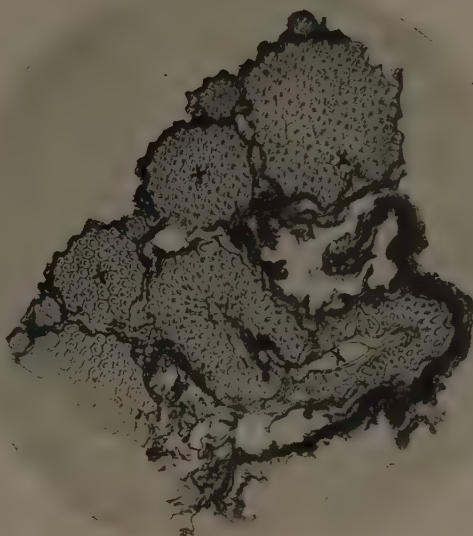
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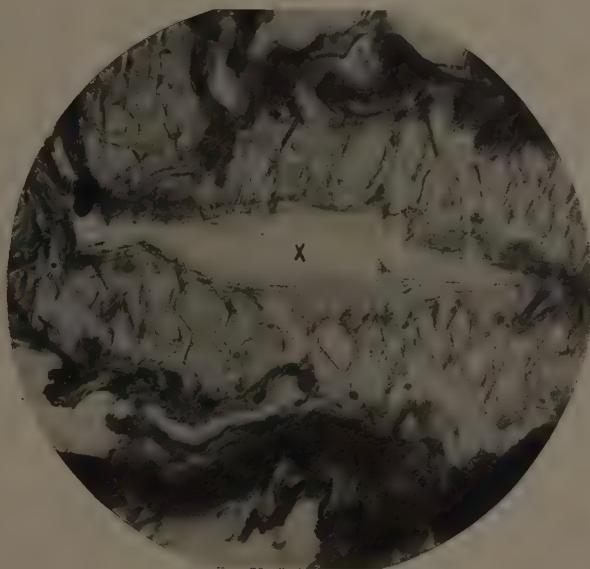
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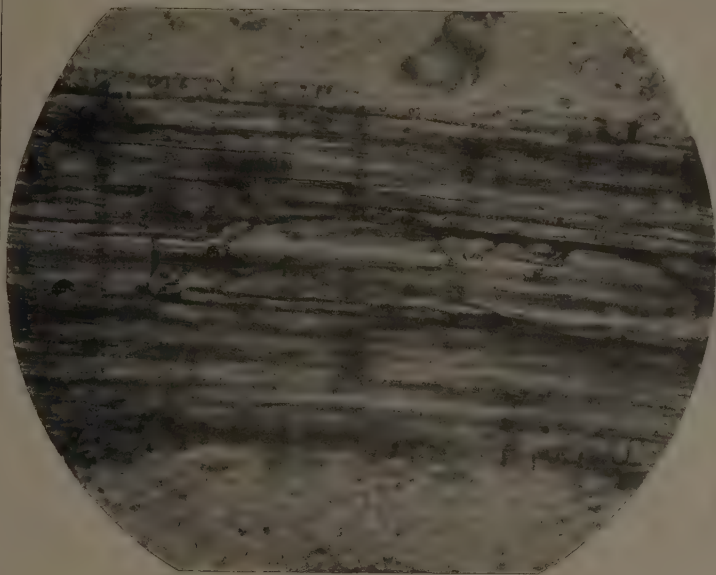
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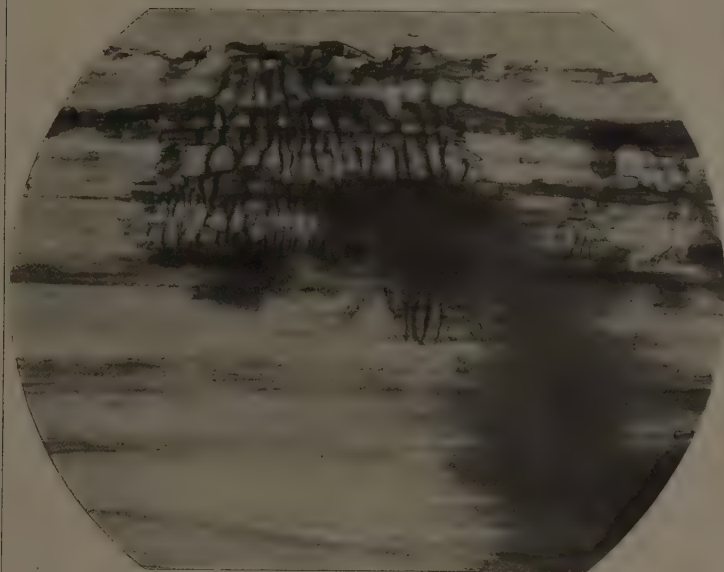
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2



1



2

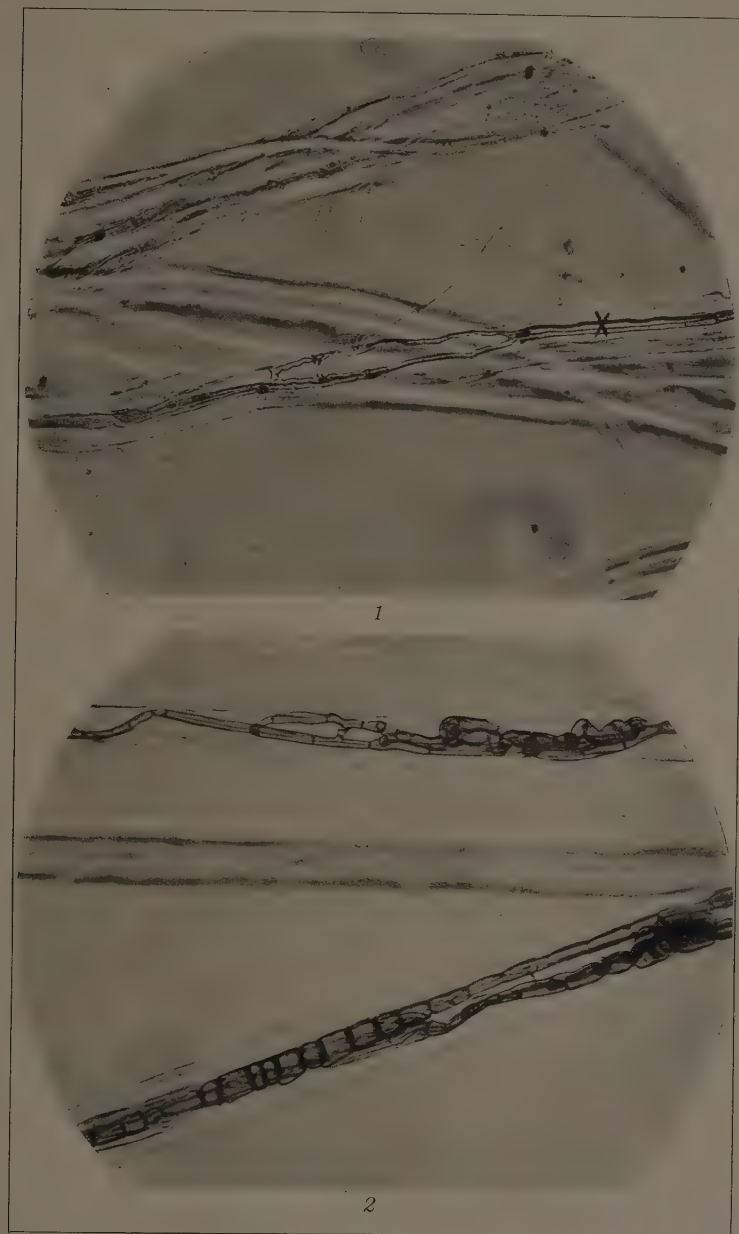


PLATE 7.

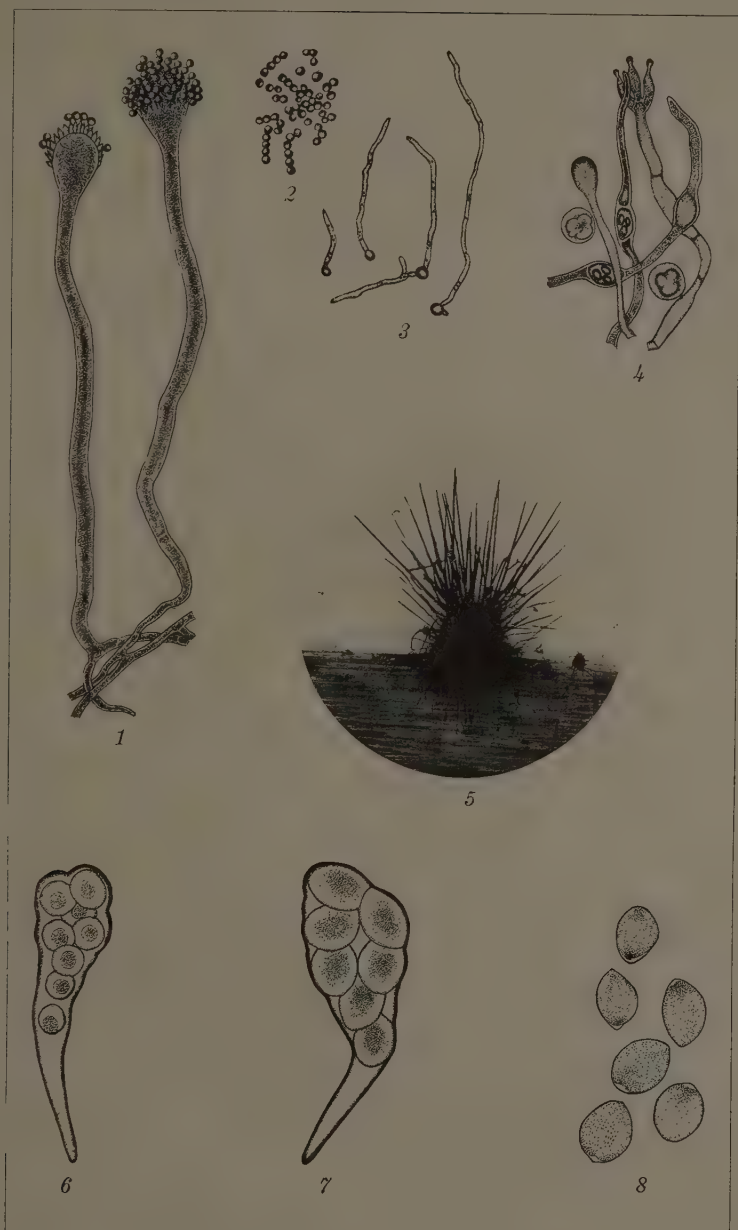


PLATE 8.



1



2



3



4



1

2

3

4

PLATE 10.

